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# INTESTINAL PARASITES ENCOUNTERED IN FIVE HUNDRED AUTOPSIES, WITH REPORTS OF CASES

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## INTRODUCTION

The incidence of intestinal parasites in the inhabitants of the Philippine Islands has been the subject of investigation by numerous workers since the American occupation of the Islands. These investigations have been made largely from the clinical standpoint, using the microscopic examination of the fæces as the standard of the incidence. Among the works on the subject may be mentioned those of Garrison,(1) Garrison and Llamas,(2) Garrison, Leynes, and Llamas,(3) Rissler and Gomez,(4) Chamberlain, Bloombergh, and Kilbourne,(5) Willets,(6) and Musgrave and Clegg.(7)

No report on any large series of cases in the Philippine Islands has been made solely from autopsy investigations with the aim of establishing the percentage incidence of intestinal parasites. The present report is based on the findings in 500 consecutive autopsies performed in the department of pathology and bacteriology, College of Medicine and Surgery, during the last twelve months. The compilation in reality entailed the examination of 583 records, 83 of which were not utilized; the excluded cases were those of infants under three months of age and those in which the intestine was not examined on account of advanced post-mortem changes or for other reasons. While the previous clinical investigations have been performed for the most part in certain selected regions or on certain selected classes of individuals, as, for instance, the male prisoners in Bilibid Prison, the present series includes all cases autopsied within a year with the exceptions already noted. There were 353 males and 147 females; 116 were below the age of fifteen and 384 were above this age.

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The autopsy service from which this report is compiled includes persons dying in a large general hospital, in a hospital for contagious diseases, in a large prison hospital, and those who were the subjects of medico-legal investigations in the city of Manila. The overwhelming majority of these cases are Filipinos, but the list also includes Americans, Europeans, Chinese, Japanese, and East Indians. The findings embodied in this report are not based on any special investigation conducted with the view to determine the absolute incidence of intestinal parasites, but have been compiled from the routine autopsy records which are on file.

In as far as our report attempts to be statistical, its interpretation should be made with certain restrictions. While, as above indicated, the class of cases on which it is based is more comprehensive than some of the series of cases previously investigated in the Islands as to age, sex, and distribution, the fact that a large percentage of these cases comes from a hospital where routine treatment for intestinal parasites is in vogue may diminish the number of parasites found post mortem and somewhat invalidate the percentage values. Also, the readiness with which *Ascaris* is recognized on account of its size and the difficulty encountered in detecting the smaller *Trichuris*, hookworm, and *Oxyuris* may have some bearing on the subject in as much as the autopsies were not performed with this investigation in view. Routine microscopic examination of the fæces for ova has not been performed by us.

The pathogenicity of some of the intestinal parasites has been the subject of so much discussion and the conclusions which have been accepted are based on such uncertain grounds that reports will be included here of certain cases where definite pathological conditions have been found, which in some cases certainly bear relation to the infesting parasite and in other cases may with a certain amount of probability be definitely associated with them.

The situation with regard to the influence of the parasites on the human host is stated by Braun (8) as follows:

> In a great many cases we are not in a position to state anything regarding any marked influence exercised by the parasite on the organism and on the conditions of life of the host. Most animals and many persons exhibit no signs of such influence. As a general rule, the parasite, which

is always smaller and weaker than its host, does not attempt to endanger the life of the latter, as simultaneously its own existence would be threatened. The parasite, of course, robs its host, but usually in a scanty and sparing manner, and the injuries it inflicts can hardly be taken into account. There are, however, numerous cases in which the situation of the parasites or the nature of their food, added to their number and movements, may cause more or less injury, and even threaten the life of the host.

For purposes of easy comparison, the tables compiled and published by Willets,(6) bearing on the parasites found by clinical examination of fæces, are inserted.

TABLE I.—*Summary of findings.*(6)

| Examinations and infections. | Number. | Per cent. |
|---|---|---|
| Persons examined | 4,278 | |
| Persons infected | 3,656 | 85.46 |
| Persons infected with— | | |
| *Ascaris* | 2,653 | 62.04 |
| Hookworm | 2,326 | 54.37 |
| *Trichuris* | 342 | 7.99 |
| *Oxyuris* | 64 | 1.50 |
| *Tænia* | 59 | 1.38 |
| *Hymenolepis* | 5 | 0.12 |
| *Strongyloides* | 4 | 0.09 |
| Trematodes[a] | 1 | 0.02 |
| Total infections | 5,454 | 127.49 |

[a] Probably *Fascioletta ilocana* Garrison, 1908.

TABLE II.—*Percentages of persons infected and total infections in various parts of Luzon.*(6)

| Authority and date. | Place. | Sex. | Examined. | Infected. | Per cent. | Total infections.[b] | Per cent. |
|---|---|---|---|---|---|---|---|
| Garrison, 1908 (1) | Manila | Mostly males | 4,106 | [a] 3,447 | 84.00 | 5,812 | 142.00 |
| Garrison and Llamas, 1909 (2). | do | Women and children. | 385 | [b] 342 | 89.00 | 533 | 138.70 |
| Garrison, Leynes, and Llamas, 1909 (3). | Taytay, Rizal | Males and females. | 1,000 | [a] 959 | 95.90 | 1,726 | 172.60 |
| Rissler and Gomez, 1910 (4). | Las Piñas, Rizal | do | 6,018 | [a] 5,406 | 89.83 | 8,996 | 149.48 |
| Do | Tuguegarao, Cagayan. | do | 2,594 | [a] 1,932 | 74.13 | 2,887 | 111.30 |
| Do | Santa Isabel, Ilagan, Isabela. | do | 802 | [a] 692 | 86.28 | 927 | 114.34 |
| Chamberlain, Bloombergh, and Kilbourne 1911 (5). | Baguio, Benguet | Adult males | 119 | [b] 110 | 92.50 | 209 | 174.00 |
| Willets, 1911 (6) | San Antonio and Maluno, Ilagan, Isabela. | Males and females. | 4,278 | [b] 3,656 | 85.46 | 5,454 | 127.49 |
| Total | | | 19,302 | 16,535 | 85.66 | 26,544 | 137.52 |

[a] Protozoan findings included.

[b] Intestinal worms only.

TABLE III.—*Comparison of the various parasites reported in different parts of Luzon.*(6)

| Authority. | Date. | Place. | Number examined. | *Ascaris.* |  | *Trichuris.* |  | Hookworm. |  | *Oxyuris.* |  | *Strongyloides.* |  | *Tænia.* |  | *Hymenolepis.* |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  | Number. | Per cent. | Number. | Per cent. | Number. | Per cent. | Number. | Per cent. | Number. | Per cent. | Number. | Per cent. | Number. | Per cent. |
| Garrison (1) | 1908 | Manila, Bilibid Prison | 4,106 | 1,052 | 26.00 | 2,426 | 59.00 | 2,135 | 52.00 | 32 | 0.80 | 132 | 3.00 | 30 | 0.70 | 5 | 0.10 |
| Garrison and Llamas (2). | 1909 | Manila, hospitals, etc. | 385 | 182 | 53.22 | 300 | 87.60 | 46 | 13.45 | 2 | 0.60 | 2 | 0.60 | 1 | 0.30 | 0 | 0.00 |
| Garrison, Leynes, and Llamas (3). | 1909 | Taytay, Rizal Province | 1,000 | 829 | 82.90 | 770 | 77.00 | 116 | 11.60 | 4 | 0.04 | 7 | 0.70 | 0 | 0.00 | 0 | 0.00 |
| Rissler and Gomez (4). | 1910 | Las Piñas, Rizal Province. | 6,018 | 4,647 | 77.21 | 3,241 | 53.40 | 671 | 11.14 | 298 | 4.95 | 135 | 2.24 | 4 | 0.06 | 0 | 0.00 |
| Do | 1910 | Tuguegarao, Cagayan | 2,594 | 1,907 | 73.55 | 672 | 25.90 | 208 | 8.01 | 68 | 2.62 | 0 | 0.00 | 13 | 0.50 | 19 | 0.73 |
| Do | 1910 | Santa Isabel, Ilagan, Isabela. | 802 | 486 | 60.59 | 50 | 6.23 | 364 | 45.38 | 10 | 1.24 | 0 | 0.00 | 11 | 1.37 | 6 | 0.74 |
| Chamberlain, Bloombergh, and Kilbourne (5). | 1911 | Baguio, Benguet | 119 | 87 | 73.00 | 72 | 60.00 | 35 | 29.00 | 0 | 0.00 | 0 | 0.00 | 15 | 12.00 | 0 | 0.00 |
| Willets (6) | .911 | San Antonio and Maluno, Ilagan, Isabela. | 4,288 | 2,653 | 62.04 | 342 | 7.99 | 2,326 | 54.37 | 64 | 1.50 | 4 | 0.09 | 59 | 1.38 | 5 | 0.12 |
| Total |  |  | 19,302 | 11,843 | 61.36 | 7,873 | 40.79 | 5,901 | 30.57 | 478 | 2.48 | 280 | 1.45 | 133 | 0.69 | 35 | 0.18 |

Our findings with reference to the more frequently encountered intestinal parasites in the series of 500 autopsies are as follows:

TABLE IV.—*Incidence of intestinal parasites in 500 autopsies.*

| Parasites. | Number of cases. | Per cent. |
|---|---|---|
| *Ascaris* | 206 | 41.2 |
| Hookworm | 83 | 16.6 |
| *Trichuris trichiura* | 172 | 34.4 |
| *Oxyuris* | 5 | 1.0 |
| *Tænia* | 1 | 0.2 |
| *Entamœba* | 25 | 5.0 |

As to other parasites we may mention the occurrence of malaria in 5 per cent of the cases, liver flukes (*Clonorchis sinensis*) in 2 cases, and at least 1 case of schistosomiasis.

Our figures are lower than the averages compiled by Willets, the incidence of *Entamœba* being especially lower than the results published by many authors. The incidence of the various parasites will now be taken up in greater detail, and reports will be added of the cases which we desire to place upon record.

## ASCARIS

Our figures (41.2 per cent) on the incidence of *Ascaris* are lower than those of Willets (6) and higher than those of Garrison. (1) The number of *Ascaris* found in any one case varied from one to nearly two hundred, and while the worms have not been individually identified they have all been classed as *Ascaris lumbricoides*. It has been stated by Braun (8) and other authors that the *Ascaris* leaves the human host in the course of febrile diseases. In order to study this question in our series, the febrile cases were studied with the following result:

| | Per cent. |
|---|---|
| 25 cases of malaria, with *Ascaris* 9 | 36 |
| 15 cases of typhoid, with *Ascaris* 4 | 26.6 |
| 46 cases of plague, with *Ascaris* 17 | 36.9 |
| 25 cases of lobar pneumonia, with *Ascaris* 14 | 56.0 |
| 14 cases of bacillary dysentery with *Ascaris* 9 | 66.6 |

This table shows that in our series the febrile cases did not have a lower percentage of infestation with *Ascaris* than the nonfebrile cases.

As has been emphasized by many clinicians and pathologists dealing with this subject, in by far the greater number of cases the individuals harboring these parasites present no derangements which can be attributed to the presence of the parasites. That they can in some cases produce harmful action, either mechanically or by wandering from the intestine to other parts of the body, has been definitely established. Their toxicity is a matter still under active discussion.

Flury(9) in a chemical and toxicological study of the horse- and pig-ascaris found numerous substances both in the body substance and in the excretions which caused local irritation or necrosis. He was able to demonstrate volatile aldehydes of fatty acids and even the free acids, the principal ones being valerianic and butyric acids, and in smaller amounts formic, acrylic, and propionic acids, alcohols and esters of the ethyl, butyl, and amyl series. All of the symptoms of irritation of the mucous membranes noted by zoölogists and the digestive disturbances in ascariasis he attributed to these substances. All of the disturbances of the central nervous system attributed to *Ascaris* (hallucination, hysteria, chorea, epilepsy, cramps, tetanus, delusions, psychic disturbances) may be explained on the basis of chronic aldehyde poisoning. A nitrogenous compound was found which caused death with severe hæmorrhages in the intestine after subcutaneous injection in the dog. This was ascribed to its action as a capillary poison. Flury concludes that *Ascaris* produces not a single poison, but a number of pharmacologically active substances which may produce very different and at times very severe symptoms.

Cases encountered in our series exemplifying the wandering of the *Ascaris* both before and after the death of the host and one where there was a possible toxic action will be recorded.

### CASE 1

*Ascaris in the vermiform appendix.*—This case came to us from the operating room of the Philippine General Hospital. A 21-year-old Filipina was operated upon to relieve symptoms due to a prolapse of the uterus. In the course of the operation the appendix was found to be enlarged and congested and was removed by the routine method, a ligature being placed about its base. When received at the laboratory the following record was made: "The specimen consists of an appendix 7.5 centimeters in length and 1 centimeter in diameter; it is stiff and blue. The superficial vessels are rather prominent. On section through the proximal end the mucosa bulges, and a small amount of thick turbid substance exudes. On opening the appendix it is found to contain a large living *Ascaris*, the body of which has been cut, apparently a small part having been left in the cæcum. The mucosa of the appendix is reddened, and shows one minute ecchymosis. Microscopic sections show some congestion of the mucosa, some fibrosis of the submucosa, œdema of the muscular and serous tunics, and infiltration of the muscularis by polymorphonuclear leucocytes and lymphocytes. Two ova of the *Ascaris* were found overlying the tissue in one section of the appendix." Examination of the fæces of this patient previous to operation had shown the presence of *Entamœba, Trichuris, Ascaris,* blood cells, and mucus. No history was given of symptoms which were interpreted as indicative of disease of the appendix.

### CASES 2 AND 3

*Ante-mortem infestation of the liver by Ascaris.*—Two cases[3] have been encountered in which numerous ascarides had passed through the bile ducts into the liver. In the first case several of the worms in the liver were surrounded by abscesses, one of which had ruptured into the peritoneum. In the second case there was abundant evidence of inflammation about the bile ducts in the liver, indicating the presence of the worms before death of the host.

### CASE 4

*Post-mortem wandering of the Ascaris.*—One case worthy of note occurred in a child, whose middle ear was found to contain a live *Ascaris* after the death of the host.

Numerous cases have been encountered in which the ascarides were found in the bile ducts, pancreatic duct, stomach, œsophagus, and larynx.

### CASE 5

*Case of possible toxic action of the Ascaris.*—This case, which occurred in our series, was reported by Albert and Mendoza at the ninth annual meeting of the Philippine Islands Medical Association. An 8-year-old Filipino, six days before death, was seized with an attack of vomiting, followed on the next day by pain in the chest and knee joints. There soon developed external œdema, jaundice, melæna, hæmorrhage from the gums, dyspnœa, prostration, and ecchymoses on the right upper eyelid and over the lower extremities. Physical examination on the day before death showed the above conditions, hæmic cardiac murmurs, and evidence of severe anæmia. Blood examination showed:

| | |
|---|---|
| Leucocytes | 11,500 |
| Polymorphonuclears | 88 per cent. |
| Small lymphocytes | 9 per cent. |
| Large lymphocytes | 2 per cent. |
| Transitionals | 1 per cent. |
| Erythrocytes | 1,080,000 |
| Hæmoglobin | 30 per cent. |

The Widal reaction with *B. typhosus* was negative, and a blood culture remained sterile.

There was no fever.

The autopsy report in full follows.

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[3] These cases will be reported in detail by Dr. V. L. Andrews.

### ANATOMIC DIAGNOSIS

Purpura hæmorrhagica; ecchymoses, cutaneous, epicardial, pleural, retroperitoneal, retropleural, gastric, and intestinal; partial atelectasis of lungs; anæmia of the viscera; hæmorrhage into intestines; ascariasis; trichuriasis; ankylostomiasis.

### BACTERIOLOGIC DIAGNOSIS

Cultures made from the spleen and the hæmorrhagic area behind the left knee joint on agar-agar, in boullion, and on blood serum remain sterile forty-eight hours.

### PROTOCOL

*Body* is that of a fairly well-nourished Filipino child, weighing 17.10 kilograms and measuring 110 centimeters in length. Rigor mortis is present. Over the right outer canthus is a minute bluish area, and there are two or three isolated, small bluish areas over the lower extremities. (These during life were bright red, ecchymotic areas.) The corneæ and conjunctivæ are clear. Ears and nose normal. The margins of the teeth are blood-stained, but the gums themselves are pale.

On section there is a small amount of subcutaneous fat. Muscle tissue is moist and brown. On stripping muscles from the thorax, the right fourth intercostal muscle near its sternal attachment shows considerable infiltration of blood. The peritoneum contains a small amount of blood-stained fluid. That covering the anterior abdominal wall is smooth and pale. The serosa of the intestine is smooth, but there is extensive subperitoneal ecchymosis over the left kidney extending on to the mesocolon and mesentery. This is also extensive in the tissues at the lesser curvature of the stomach and along the vertebræ surrounding the abdominal aorta. Diaphragm at the fourth interspace on the right, fifth interspace on the left. On removal of the sternum, the tissues on its under surface show extensive ecchymosis. Lungs are considerably retracted, there being some blood-tinged fluid in both pleural sacs. The pericardium contains some excess of clear fluid. The structures of the neck and thorax were removed *en masse.*

*Thorax; organs of neck.*—The tongue is blood-stained on its superior surface throughout its anterior half. Tissues at the base of the tongue are not blood-stained, and the lymphoid tissue is not unduly prominent. Tonsils are of normal appearance. Œsophagus, larnyx, and trachea are normal. Surrounding the trachea and œsophagus in the posterior mediastinum, the tissues are infiltrated with blood to an area of about 2 centimeters peripherally from the œsophagus in all directions. Thymus gland is small, and contains very abundant fat.

*Heart.*—There are numerous minute ecchymoses in the epicardium. The heart itself contains fluid blood. Its myocardium and endocardium are intact. It is of about normal size, and the musculature is extremely pale. Heart weighs 85 grams.

*Lungs* are retracted and of small volume, the right lower lobe being adherent to the tissues about the vertebræ at the extreme lower portion, that is, near the median line. The lungs present numerous, dark, depressed, firmer areas which have regular outlines, and the lower portion of the right lower lobe which was adherent along the median line is still firmer than these areas. On section of the lungs they are pale, except for the firmer areas which are darker and contain practically no air, being rather leathery in consistence. The portion at the lower part of the right lower lobe on section cuts readily and is made up of an intensely hæmorrhagic mass in which no structure can be differentiated. Peribronchial nodes are not enlarged. The larger pulmonary vessels are free.

*Spleen* is not enlarged and has a normal appearance, except for marked pallor. The lymphoid tissue is not prominent. Spleen weighs 42 grams.

*Adrenals* are normal in size and appearance.

*Kidneys* are imbedded in a small amount of fat. Ecchymosis over the left kidney has already been referred to. Kidneys are of normal size. On stripping the capsules, minute hæmorrhages are visible on the internal surface of the capsules. The surfaces are smooth and very pale. On section they are of normal appearance except for very marked pallor. The pelves and ureters are free. Both kidneys weigh 101 grams.

*Urinary bladder* is considerably distended with clear urine. Its mucosa is pale and smooth.

*Intestine.*—There are very numerous ascarides in the small intestine and stomach. These number about 150. A few ankylostomata and many trichurides are also present. There is considerable blood-stained fluid throughout the intestine and numerous ecchymoses, especially in its upper portion.

*Stomach* contains some ascarides and few ecchymoses on its mucous surface.

*Pancreas* is practically surrounded by hæmorrhagic tissue, but on section is of normal size and appearance, except for very marked pallor.

*Gall bladder* and bile ducts are normal.

*Liver* is of about normal size with a thin capsule, and on section the cut surface is smooth, showing rather prominent bile ducts. The color is pale yellowish brown. Lobular markings are fairly distinctly visible. Liver weighs 458 grams.

Along the vertebral column, extending through the diaphragm at the œsophageal opening and continuing retroperitoneally to the bifurcation of the aorta, is a very extensive hæmorrhagic mass which is firm, 2 to 3 centimeters in radius, its center being about the aorta. The mesenteric lymph nodes are enlarged, pale, and firm.

Structures of the scalp and calvarium are normal. Dura mater is adherent to the inner surface of the calvarium. There is no excess of fluid in the meninges over the hemispheres. The brain itself is firm and very pale.

On section into the right knee joint, it is found to contain clear fluid, and there is one minute petechia anteriorly in the capsule of the joint. On section into the left knee joint, it is also found to contain clear fluid, and all the muscle tissue posterior to the joint shows extensive hæmorrhagic infiltration.

## TRICHURIS

Our figures (34.4 per cent) on the incidence of *Trichuris* are a little below the average (40.79 per cent) established by Willets(6) in his compilation of the statistics of investigators in the Philippine Islands, but are higher than those of three of the investigators. Our records in this case probably represent the true incidence of the condition in the cases examined by us, as these worms are notably extremely difficult to dislodge by medication.

In our autopsy series no instance has occurred where any pathological condition was encountered which could be traced to *Trichuris* even though very large numbers of the parasites have been found in some of the cases. They are not infrequently attached rather intimately to the mucous membrane of the cæcum, and the sole exception to the above statement of the absence of pathological conditions has been the occasional finding of minute petechiæ in the mucosa of the cæcum which have been attributed to *Trichuris*. However, this relation has not been established by serial microscopic sections.

On account of the frequent assertion that *Trichuris* is more prevalent in females and children than in males and adults, our records were examined to ascertain the incidence in our series with respect to sex and age. The following record shows our findings.

TABLE V.—*Incidence of trichuriasis according to age and sex.*

| | Total males. | Males under 15 years. | Males over 15 years. | Total females. | Females under 15 years. | Females over 15 years. | Total under 15 years. | Total over 15 years. | Total. |
|---|---|---|---|---|---|---|---|---|---|
| Cases examined | 353 | 78 | 275 | 147 | 38 | 109 | 116 | 384 | 500 |
| Cases trichuriasis | 120 | 14 | 106 | 52 | 4 | 48 | 18 | 154 | 172 |
| Per cent trichuriasis | 33.9 | 17.9 | 88.5 | 35.4 | 10.5 | 44.0 | 15.53 | 40.1 | 34.4 |

Examination of this table shows that in our series the occurrence of *Trichuris* in females is very little more frequent than in males, and that the cases over 15 years of age had the parasites nearly three times as frequently as those under that age.

Metchnikoff(10) was the first to ascribe a rôle to *Trichuris* in the etiology of appendicitis, and the literature on the whole subject of trichuriasis up to 1908 had been carefully reviewed by Musgrave, Clegg, and Polk,(11) who say that "very little is given about the clinical findings, but *a priori* there is no reason to assume that they would be materially different from appendicitis of other etiology."

Castellani and Chalmers(12) in discussing the appendicular variety of trichuriasis say "the symptoms of this variety are the same as those for appendicitis arising from other causes. Operative treatment reveals the nature of the malady."

Cecil and Bulkley(13) have reported finding trichurides in two cases of appendicitis, 1 catarrhal in type and 1 gangrenous.

The establishment of direct etiological relationship seems rather arbitrary, and the pathological changes described by them do not seem pathognomonic of trichuriasis.

Among the very large number of appendices received from the surgical clinic, 2 have been examined which contained *Trichuris.* The total number of cases examined is not given for the reason that not all were opened before fixation. However, microscopic sections of none of the appendices showed anything which led to the suspicion of the presence of *Trichuris,* and in the two cases mentioned the presence of *Trichuris* would not have been suspected from the sections made.

### CASE 1

*Trichuris in the vermiform appendix.*—A 21-year-old Filipino was admitted to the hospital with symptoms pointing to disease of the appendix and giving a history of two previous similar attacks within four months. The symptoms were some pain over the region of the appendix, tenderness on slight pressure, nausea, some vomiting, and slight rigidity. The temperature was not above 38°.6 C. before operation. He was in the hospital for twenty-six days before operation; his leucocytes, which had ranged from 12,600 to 14,800 per cubic millimeter of blood previous to this, numbered 28,000 on the day of operation. At the operation the appendix was removed, and it was noted that there were adhesions about the intestines. The examination of the fæces previous to operation was unsatisfactory, but 3 examinations at varying periods after operation showed the presence of the ova of hookworm but none of *Trichuris.*

When received at the laboratory the following record was made of the appendix: "The specimen consists of an appendix which is 6 centimeters long and has some attached mesenteric fat. It is about 7 millimeters in diameter, and the vessels of the serosa are prominent. On opening the appendix, it is found to contain a small amount of soft brown fæcal material, and the mucosa is somewhat swollen and moist, and shows a few injected areas. Two trichurides are found in the lumen, rather closely attached to the mucosa. Microscopically there is seen some loss of epithelium with the passage of lymphocytes into the lumen, and the epithelium of the glandular tubules shows a well-marked catarrhal state. There is active proliferation of the lymph follicles and some fibrosis of the submucosa. Marked round-celled infiltration of the submucosa is seen as well as of the outer muscular and serous coats. The outer muscular coat is very œdematous, and the vessels just beneath the serosa are much dilated and surrounded by dense masses of round cells. Eosinophiles in large numbers are present throughout all parts of the sections."

### CASE 2

*Trichuris in the vermiform appendix.*—In the course of a gynæcological operation on a 38-year-old Filipina the appendix was removed. It was a small, somewhat distorted appendix, from the cut proximal end of which an intact *Trichuris* protruded. Microscopic examination of this appendix shows a very thin muscular tunic and an intact serosa. The lymphoid tissue is scanty. In the mucosa there is some congestion and some leucocytic infiltration and œdema, the cells of the glandular tubules being largely converted into goblet cells. At the point where the *Trichuris* was in contact with the mucosa several ova are found in the crypts of the mucosa.

## HOOKWORMS

In considering the incidence (16.6 per cent) of hookworms in our series, it must be borne in mind that the majority of our cases are derived from a hospital where careful examination of the fæces is a routine clinical procedure and active treatment of this disease is the rule. Willets' percentage based on the work of different investigators in the Philippine Islands was 30.57, while his own clinical examination of 4,288 cases gave him a percentage of 54.37.

Chamberlain,(14) as a result of his own investigations, concludes that "Uncinariasis is found among the Filipinos in probably not over 15 per cent of the general population and is mild in type and of small economic importance."

While anæmia, the chief symptom in hookworm disease, has been a prominent feature in a large number of our cases, no case has been encountered in which the hookworm was considered an important factor, and the death of the patient has always been ascribed to other causes. However, a not infrequent finding in our cases has been punctate hæmorrhages in the mucosa of the upper part of the small intestine sometimes with slight erosion or, if not actual hæmorrhages, minute pigmented spots. These latter have been interpreted as evidence of residual blood pigment from previous hæmorrhage. One case which exemplifies the condition we have encountered, and in which the number of hookworms found was unusually great, is here reported. In this case the bacillary dysentery was the cause of death, and the previous malarial infection along with the dysentery may have accounted for the severe anæmia. Nevertheless, neither of these caused the condition described in the small intestine, and the case does serve to illustrate our findings, at the same time emphasizing the presence of other diseases as being the ones to cause death.

### CASE REPORT

A 24-year-old male Japanese was admitted to hospital four days before death with symptoms of bacillary dysentery, marked anæmia, and an enlarged spleen. The temperature range was between 37°.5 and 39°.5 C. Pulse 110 to 140. Fæces contained ankylostoma ova, blood, mucus, and pus. The urine contained a trace of albumin and numerous casts. The blood was not examined. At autopsy the following anatomical diagnosis was made:

Acute colitis (bacillary dysentery); acute lymphadenitis, mesocolic; subacute splenitis, malarial; infarcts of spleen; acute parenchymatous degeneration of liver and kidney; malarial pigmentation of liver, pancreas, and lungs; congestion and œdema of lungs; hookworms; and hæmorrhages and erosions in mucosa of small intestine.

*Bacillus dysenteriæ* was isolated from the mucosa of the large intestine.

The description given of the intestines was as follows:

In the duodenum are found a few hookworms, one being attached within 5 centimeters of the pylorus. In the jejunum they are found in great numbers both attached and free. At the points of attachment of many, and also between them, are small hæmorrhagic areas which are sometimes visible from the serous surface. Over a number of these there occur shallow erosions of the mucosa. In the lower 10 centimeters of the ileum there is marked hyperæmia of the mucosa. The mucosa of the ileocæcal valve is swollen and reddened. Throughout the large intestine the mucosa is greatly swollen, œdematous, and hyperæmic. The greatest congestion occurs in the ascending and sigmoid portions. In many places small ulcers have formed, most frequently on the tips of the folds of the mucosa. The swelling and congestion extends into the appendix.

Sections taken through the areas in the small intestine mentioned as hæmorrhagic show a destruction of epithelium and glandular tubules. The mucosa and submucosa immediately beneath are œdematous, and many leucocytes and erythrocytes are present. Extending for a short distance each way from this, situated deep in the submucosa, there is a thin layer of extravasated blood.

The lesions in the small intestine in this case can with a very great degree of probability be ascribed to the hookworms, while the lesions of the large intestine are those of bacillary dysentery.

## OXYURIS

The incidence (1 per cent) of *Oxyuris* has been so small and its presence is of so little pathologic importance that no further comment will be made upon it. It is possible that it may have been overlooked in a number of our cases.

## ENTAMŒBA

Our figures on the incidence of *Entamœba* (5 per cent) refer only to those cases in which there was a recognizable amœbic colitis. Routine examination of the fæces at autopsy has not been performed. Musgrave and Clegg(7) found 26 per cent of 587 individuals harboring amœbæ, from an examination of the fæces, and Garrison(1) found 23 per cent in 4,106 prisoners. These authors did not differentiate between pathogenic and nonpathogenic amœbæ, and according to Walker(15) individuals may harbor amœbæ without suffering from amœbic colitis.

## TÆNIA

Only one case has been encountered in which a *Tænia* was found. This occurred in a 57-year-old Filipino who died as the result of cerebral hæmorrhage dependent on arteriosclerosis. This adult worm was found in the intestine and was identified as *Tænia saginata.* Three other tapeworms obtained from the human host which are in the museum of this department are also *Tænia saginata.*

No example of the adult of *Tænia solium* has been encountered, but one case of widespread infestation with *Cysticercus cellulosæ* occurred in a 28-year-old Filipino, a resident of Manila, who died suddenly.

Cysticerci in hogs are a very frequent finding in the Philippine Islands, and these, on account of their occurrence in this host and on account of their scolices bearing 4 suckers and a rostellum with hooklets, are presumably *Cysticercus cellulosæ.* This would seem to presuppose the frequent presence of the adult parasite in the human host. However, the adult worm has not been found in our series, and only one example of *Cysticercus cellulosæ* has occurred in a series of over 2,200 autopsies performed in this department.

The autopsy report of our case will be given in full.

### ANATOMIC DIAGNOSIS

*Cysticercus cellulosæ* of brain, hypophysis, and muscles; dilatation of right ventricle; congestion of lungs; passive congestion of liver and kidneys; chronic fibrous pleurisy; persistent thymus; hookworms; ascariasis; trichuriasis.

*Body* is that of a large, well-developed and fairly well-nourished Filipino, weighing 55.65 kilograms and measuring 172 centimeters in length. Post-mortem suggillation is marked on the back. There are no external evidences of violence. Left knee is slightly flexed. Rigor mortis is marked. The body is twisted slightly to the right above the pelvis.

A small elevation is noticed on the left arm over the biceps muscle; this feels firm, and the skin moves freely over it. On incising the skin there is found just beneath the fascia of the biceps muscle a small, oval, translucent cyst, measuring 15 millimeters in length by 6 millimeters in thickness. The long axis is parallel to the muscle fibers. The capsule of the cyst is thin but moderately resistant. Another cyst is found near this, and others are found just beneath the fascia of other muscles, one in the right biceps, one in the left extensor digitorum communis, one in the lateral head of the left gastrocnemius, and one in the subscapularis. Most of these are somewhat smaller than the one described, particularly in the long diameter. The deeper muscles were not investigated. Subcutaneous fat is present in moderate amount.

*Peritoneum* is smooth and pink. The peritoneal cavity contains a small amount of clear, light-reddish fluid. Appendix appears to be normal.

There is a slight fibrous adhesion between the colon and the inferior surface of the liver. Diaphragm stands at the fifth interspace on the left side and the fourth interspace on the right.

*Thorax.*—The pericardial sac is large, and contains a few cubic centimeters of clear fluid. The thymus is long and narrow, measuring 12 by 4 centimeters. It is pink, both externally and on section. Thymus weighs 30 grams.

*Lungs.*—Left lung is bound to the chest wall by a few fibrous adhesions. The bronchi are pink, and contain a little frothy fluid but no mucus. On section the lung is slightly moist, but on pressure very little serous fluid can be expressed. However, the blood vessels are well filled with dark fluid blood. Its surface is smooth, except near the anterior border where there is a fibrous tag. Color of the surface is purplish red. The lung is everywhere soft and crepitant, and pits slightly on pressure. On section the right lung is similar to the left, although the surface is slightly more moist.

*Heart.*—On the anterior surface of the right ventricle are 2 slightly thickened, grayish patches. The right ventricle is very prominent, but the left also seems large. The tricuspid ring admits easily four fingers. The auricle is slightly larger than normal. The right ventricle is markedly dilated; the wall, however, is thin, measuring 3 to 5 millimeters in thickness and the muscle is soft. The tricuspid ring measures 13 centimeters in circumference. The left auricle is normal in size. The left ventricle is fairly well contracted, the chamber being small, and the muscle rather soft and light reddish grayish brown in color. The papillary muscles are rather thick. The wall measures 15 millimeters in thickness. The mitral ring measures 10.5 centimeters. Pulmonary ring measures 7.2 centimeters. Heart weighs 353 grams.

*Spleen* is of normal size. On section it is found to be rather firm. The surface is dark red, and the organ contains considerable blood. The Malpighian bodies are barely visible. Spleen weighs 187 grams.

*Kidneys* are large. The capsules strip easily, leaving a smooth, purplish red surface. On section the cortex is bluish red in color; the glomeruli are barely visible; striations are regular. The pyramids are purple. The organs are moderately firm. Both kidneys weigh 272 grams.

*Adrenals* are thin, and the medullary substance is scanty.

*Pancreas* is rather firm. On section it is pink.

*Liver* is enlarged. The surface is smooth, and the organ is fairly firm. On section it is uniformly dark red in color and friable, the lobules being easily seen. Liver weighs 1,815 grams.

*Stomach* contains a large amount of partially digested food consisting principally of rice.

*Intestines.*—There is slight hyperæmia of the upper part of the small intestine, otherwise no lesions are noted. Intestines contain hookworms, ascarides, and trichurides.

*Genitalia* are rather small but otherwise normal.

*Urinary* bladder is normal.

*Brain.*—Meninges are rather moist, especially about the base where they seem slightly thickened. On the surface of the brain in, or in most cases beneath, the pia-arachnoid and embedded in the cortex are found a number of small cysts. These have a slightly thicker capsule, and are in most cases more nearly spherical than those of the muscles. Some lie upon the gyri, while others are deep in the sulci. On the left hemisphere, one is found on the superior frontal, one on the middle frontal, one on the posterior central gyrus, one on the temporal pole, two on the middle temporal gyrus, one on the external surface of the occipital lobe, near the anterior end of the occipito-temporal gyrus, and one on the precuneus. On the right hemisphere one is found on the inferior frontal, two on the inferior temporal, one in the collateral sulcus, and one in the lateral occipital sulcus. There is one also on the right precuneus. On section of the brain, one is found on the left lateral wall of the third ventricle, projecting slightly into its lumen. The lateral ventricles are not dilated; the cerebrospinal fluid is clear. On section of the basal ganglia one cyst is found in the lower lateral portion of the right thalamus and one in the head of the right caudate nucleus. On removing the hypophysis, a small cyst similar in appearance to those of the brain but containing soft, gelatinous material is found on its inferior surface.

### ADDENDUM

Closer inspection of a number of the cysts reveals the following: Near the middle is seen a white spot which, on opening the cyst, is seen to correspond with a small rounded mass projecting from one side into the lumen. Under the microscope this projection resembles the head of a tapeworm. There are 4 suckers beyond which is a circular row of hooklets, 26 in number.

## CLONORCHIS

Two cases have occurred in which liver flukes were found. In the first case they were present in the cystic, common, and intrahepatic bile ducts of a 27-year-old Chinaman who died of an acute dilatation of the heart, complicating chronic nephritis.

The second case in which they occurred was also a Chinaman, 29 years of age, who had died as the result of a perforation of the intestine in the course of typhoid fever. In this case they were found only in the intrahepatic bile ducts. In neither case did the bile ducts or liver show any changes attributable to the presence of the parasites.

The flukes in both cases have been identified by Doctor Willets of the Bureau of Science as *Clonorchis sinensis* Cobbold, 1875.

The identification is based upon the presence of dark-brown, granular pigment in the body parenchyma and the excretory apparatus and the following anatomical characters:

*Size and shape.*—Flat; elongated, 18 by 3.5 millimeters; another, 18 by 4 millimeters.

*Cuticular spines.*—Absent.

*Anterior end.*—Rather pointed. Head cone absent.

*Oral sucker.*—Small.

*Acetabulum.*—Small; well developed; near oral sucker.

*Pharynx.*—Small; well developed.

*Œsophagus.*—Rather short.

*Intestinal cæca.*—Unbranched; extending to near the posterior extremity of the body.

*Excretory vesicle.*—Tubular; contains considerable, fine dark-brown, granular pigment.

*Testes.*—Two; posterior; branched with irregular swellings.

*Receptaculum seminis.*—Very well developed; relatively large; immediately anterior to anterior testis.

*Ovary.*—Lobate; anterior to receptaculum seminis.

*Uterus.*—Full of yellowish brown, operculated ova; between acetabulum and ovary.

*Genital pore.*—Mid-line; immediately anterior to acetabulum.

*Vesicula seminalis.*—Present; well developed; long.

*Cirrus pouch.*—Absent.

*Yolk glands.*—In lateral fields; extend from about the level of the acetabulum to about that of the ovary; several follicles are underdeveloped in each set.

*Ova.*—Small, yellowish brown, operculated; average size, 30 by 16 microns.

### SCHISTOSOMA

One case[4] of schistosomiasis has been encountered in an 18-year-old Filipino. The case showed the characteristic changes, and the parasite has been identified as *Schistosoma japonicum.*

### SUMMARY

In a series of 500 consecutive autopsies on people of all ages in Manila:

*Ascaris lumbricoides* occurred in 41.2 per cent, *Trichuris trichiura* in 34.4 per cent, hookworm in 16.6 per cent, *Tænia saginata* in 0.2 per cent, *Cysticercus cellulosæ* in 0.2 per cent, *Oxyuris* in 1 per cent, *Clonorchis sinensis* in 0.4 per cent, *Schistosoma japonicum* in 0.2 per cent, malaria in 5 per cent, and amœbic colitis in 5 per cent.

The manifestations of ascariasis have been the presence of *Ascaris* in the liver as the result of both ante-mortem and post-mortem wanderings, and its possible action as the exciting factor in one case of widespread hæmorrhages. One clinical case of *Ascaris* in the appendix has been encountered. *Ascaris* has not been found less frequent in febrile than in other cases.

Trichuriasis occurred in 33.9 per cent of the males and 35.4 per cent of the females; 15.53 per cent of the cases under 15 years of age and 40.1 per cent of the cases above that age harbored *Trichuris.* Two clinical cases of appendicitis in which *Trichuris* was found in the appendix have been encountered.

One case has been described exemplifying the frequently encountered hæmorrhages in the submucosa of the intestine associated with the presence of the hookworm.

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[4] This case will be reported in full by Dr. V. L. Andrews.

One *Tænia saginata* was the only tapeworm encountered. The infrequence of *Tænia solium* in human cases as compared with the great frequence of *Cysticercus* in hogs in the Philippines has been deemed noteworthy.

One case of *Cysticercus cellulosæ* in the human cadaver has been reported in detail.

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# CONCERNING THE BERIBERI-PREVENTING SUBSTANCES OR VITAMINES CONTAINED IN RICE POLISHINGS

### A SIXTH CONTRIBUTION TO THE ETIOLOGY OF BERIBERI[1]

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It has been claimed at various times that beriberi has resulted from the use of an undermilled rice which had been stored for some time or which had been kept in a damp place. Thus Braddock(1) says:

> I have had an extensive experience with beriberi, and never saw it except when the rice used for food had been stored in a damp place; when care was taken to store the rice in a dry place, there was no beriberi.

An interesting case was reported by Bréaudat,(2) where there were two neighboring settlements. In the first the rice was always polished by hand daily, and not a single case of beriberi has ever been reported. In the second settlement the rice was polished every ten or twelve days and stored in wooden boxes, and beriberi occurred every season with an incidence of from 60 to 90 cases. He advised the manager to use rice polished by hand daily, and not a single case occurred after that procedure was adopted. Bréaudat says: "Therefore I come to the conclusion that even hand-polished rice, if 10 or 12 days old, may produce Beriberi." These statements cast grave doubts on the protective value of stored rice, particularly as it is well known that the protective substances in rice polishings are readily destroyed by certain agencies; for example, moist heat and strong alkalies.

In view of the practical importance of this question, an experiment was performed to test the keeping qualities of undermilled rice.

*Experiment 38.*—A sack of the undermilled rice furnished the Philippine Scouts was obtained from the commissary. This rice

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[1] Read before the Manila Medical Society, April 7, 1913, and published with the permission of the Chief Surgeon, Philippine Department.

[2] Edward B. Vedder, captain, Medical Corps, United States Army, member of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.

was then stored for one year in a tin can without any cover, in the ground floor of a building not specially protected from dampness. It will thus be seen that this rice was exposed to the dampness throughout an entire rainy season and was freely accessible to roaches, weevils, and other insects. At the conclusion of this period all the rice was musty, and actually moldy in spots, and absolutely unfit for human consumption.

Four fowls were now fed exclusively on this rice. All four fowls remained in perfect health for a period of four months when the experiment was discontinued. The fowls fed on this rice did not lose their appetite as do fowls fed on polished rice, but ate every grain of their ration up to the very last. It is believed that this experiment imposed a far more severe test on the keeping qualities of undermilled rice than will ever be required in the case of rice used for human consumption. Before the experiment was concluded, this rice was fully a year and a half old, since it must have been several months old before it was obtained from the commissary. This experiment shows conclusively that an undermilled rice does not lose its protective power because of dampness or long storage. We might add that in a former communication(3) we stated that we had confirmed Shiga's experiment that undermilled rice which had been allowed to ferment in the incubator still retained its protective properties, and that we have found that an extract of rice polishings, prepared according to the method previously described and kept in an ice box to prevent decomposition,(4) has retained its protective and curative powers for a period of at least seven months.

Soon after describing the preparation of this extract(4) we had reason to believe that all of the protective substances in the polishings were not extracted by this method because of their slight solubility in cold 95 per cent alcohol. Therefore, in all our later work we extracted the polishings three times with successive portions of fresh 95 per cent alcohol, using 3 liters of alcohol to each kilogram of polishings for the first extraction and 1.5 liters of alcohol for each of the two following extractions. The extract so obtained was combined. It was found, in fact, that this successive extraction with increased quantities of alcohol increased the protective and curative action of the extract.

It will be remembered that Strong and Crowell(5) used extract prepared according to our method together with a polished rice in feeding one group of men in their experiment, but that these men were not fully protected, and some of them developed symptoms of beriberi. Since Strong and Crowell extracted each 5 kilograms of polishings with 14 liters of 95 per cent alcohol in three successive macerations, while we have for some time been using 30 liters of alcohol to extract each 5 kilograms of polishings, it seemed possible that the extract used by Strong and Crowell was only partially successful in preventing beriberi because insufficient alcohol was used completely to extract the protective substances from the polishings. In order to obtain more definite information on this point, the following experiment was performed.

*Experiment 39.*—Five kilograms of polishings were extracted with 15 liters of 95 per cent alcohol. The polishings were pressed, and the dry residue again extracted twice in a similar manner, using 8 liters of fresh alcohol each time. The extracts obtained from these three processes were kept separate, the alcohol evaporated off, the fat removed, and the residue diluted with water according to our usual method.

(*a*) Four fowls were fed on polished rice plus the substances obtained on the first extraction. An amount equivalent to 10 grams of polishings was given each fowl daily. All 4 fowls remained well for a period of three months when the experiment was discontinued.

(*b*) Four fowls were fed on polished rice plus the substances obtained on the second extraction. An amount equivalent to 10 grams of polishings was given each fowl daily. All 4 fowls remained well for three months when the experiment was discontinued.

(*c*) Four fowls were fed on polished rice plus the substances obtained on the third extraction. An amount equivalent to 10 grams of polishings was given each fowl daily. All 4 fowls remained well for three months when the experiment was discontinued.

(*d*) Four fowls were fed on polished rice plus a daily dose of 10 grams of the polishings that had been extracted three times with alcohol. All 4 fowls remained well for a period of three months when the experiment was discontinued.

This experiment shows that even three successive extractions with 95 per cent alcohol, using a total of 6 liters of alcohol to each kilogram of polishings, are insufficient to remove *all* of the protective substances from rice polishings. We may, therefore, conclude that the protective substances are only slightly soluble in cold 95 per cent alcohol, and that it is very probable that the extract used by Strong and Crowell in the experiment already quoted failed to confer complete protection because the polishings were incompletely extracted by the quantity of alcohol they used.

The method of extracting by alcohol was originally adopted by us because it produced an extract that was chemically much simpler than that obtained by other methods and thus afforded a better opportunity for identifying the protective substances, and we have continued to use this method in preparing extracts for the treatment of beriberi cases in spite of the disadvantage of the comparative insolubility of the protective substances in alcohol, because we have found that extracts obtained in several other ways *are distinctly poisonous,* a fact to which we shall refer again later. We have found, however, that a more powerful extract may be obtained by using alcohol of only 90 per cent strength, or by using alcohol at a temperature of from 60° to 70°.

In a previous paper(3) we stated that sodium hydroxide destroyed the neuritis-preventing substance. We have also found that it is destroyed by ammonia.

*Experiment 40.*—In the course of our experiments to determine the solubility of the protective substances in ether, extract of rice polishings which had previously been tested and found active was rendered strongly alkaline with ammonia, and was then extracted by shaking with ether. The ether was then removed and evaporated at a low temperature and the residue diluted with water.

(*a*) Four fowls were fed on polished rice, and in addition were given daily a quantity of the substances extracted by the ether, equivalent to 10 grams of polishings. One fowl developed neuritis in twenty-eight days and one in twenty-nine days, when the experiment was discontinued.

(*b*) Four fowls were fed on polished rice, and in addition were given daily 10 cubic centimeters of the aqueous extract of rice polishings remaining after the extraction with ether. One fowl developed neuritis in thirty-eight days and one in forty-five days, when the experiment was discontinued.

Since this extract originally protected fowls completely, while, after treatment with ammonia, both the ether extract and the residual portion failed to protect, it appears that the protective substances were destroyed by the ammonia. The significance of this fact will appear later in discussing the action of barium hydroxide.

In one of his papers(6) Funk stated that while allantoin had no effect in curing pigeons that had already developed neuritis, it appeared to prolong the life of the birds. As we had found (see experiment 46) that the administration of a mixture of purine bases undoubtedly exerted some protective influence, we tried a feeding experiment with allantoin.

*Experiment 41.*—Five grams of allantoin were dissolved in 1,000 cubic centimeters of water. Four fowls were fed on polished rice and in addition were given daily 10 cubic centimeters of this solution of allantoin or 50 milligrams daily. One fowl developed neuritis in twenty days, one in twenty-five days, and one in twenty-nine days.

From this experiment it is concluded that allantoin does not possess any protective action.

Having used basic lead acetate as a precipitant in a previous experiment, (3) the result of which was in doubt, we performed the following experiment.

*Experiment 42.*—A quantity of extract of rice polishings was precipitated by basic lead acetate. The precipitate was separated from the filtrate and the lead was removed from both by hydrogen sulphide.

(*a*) Four fowls were fed on polished rice, and in addition were given a daily dose of the substances precipitated by basic lead acetate equivalent to 10 grams of polishings. One fowl developed neuritis in twenty-six days, one in thirty days, and one in thirty-two days, when the experiment was discontinued.

(*b*) Four fowls were fed on polished rice, and in addition were given a daily dose of the substances not precipitated by basic lead acetate equivalent to 10 grams of polishings. One fowl developed neuritis in thirty days, but the remaining 3 fowls continued in good health for sixty days, when the experiment was discontinued.

From this experiment it is concluded that the neuritis-preventing substance is not precipitated by basic lead acetate, but remains in the filtrate. However, since one of the fowls (probably highly susceptible to this disease) fed on this filtrate developed neuritis, it appears that a portion of the neuritis-preventing substances is lost as a result of the chemical manipulation. After we had performed this experiment, we received the paper of Edie, Evans, Moore, Simpson, and Webster(7) in which they report obtaining a similar result after using lead acetate in isolating the antineuritic substances from yeast.

Before any of Funk's papers had reached us, we had tried(3) precipitating our extract with phosphotungstic acid and had decomposed the phosphotungstates with barium hydroxide. We did not succeed in obtaining any protective action from these decomposed phosphotungstates. Funk's work, whose essential accuracy we could not doubt, was therefore apparently contradictory to our own results. After receiving Funk's papers, we repeated the feeding experiment several times, using Funk's method on our extract as we had always prepared it. In each case the result was a failure, for the fowls developed neuritis, both in the group receiving the filtrate from phosphotungstic acid and in the group receiving the decomposed phosphotungstates.

Being convinced that Funk's work was correct, and that our previous work was also correct, it became certain that, if an adequate explanation of this apparent discrepancy could be afforded, further progress would be made. This explanation we are now prepared to make. Funk(8) tried only curative experiments, while we were relying on feeding experiments. Further, Funk had extracted his rice polishings with alcohol containing 2.5 per cent of gaseous hydrochloric acid, while the preparation with which we worked was extracted by alcohol alone. These differences in method which may appear trifling are in reality crucial.

The therapeutic action of these two extracts is totally different. Funk reported that large doses of his extract were poisonous, but that, if doses small enough to avoid this poisonous action were administered to pigeons that had developed polyneuritis as a result of rice feeding, the birds were immediately cured. Our extract, on the other hand, was never poisonous, although given in enormous doses, equivalent to several kilograms of polishings; nor did it produce an immediate cure when given to birds that had already developed polyneuritis; but if the birds were given small doses of this extract daily (equivalent to 10 grams of polishings), their lives were saved, although they remained paralyzed. Further, if the administration of our extract was continued for several months, the paralysis also was thereby cured. Funk's extract was therefore poisonous, but smaller doses promptly cured the paralytic symptoms, while our extract was nonpoisonous and only slowly curative. It appeared probable that this difference in therapeutic action resulted from the fact that Funk used hydrochloric acid in the preparation of his extract while we did not. In order to test this hypothesis, the following experiment was performed.

*Experiment 43.*—A quantity of our extract was divided into two portions. One part was untreated and the other part was mixed with 5 per cent hydrochloric acid and allowed to stand at room temperature for twenty-four hours.

(*a*) A normal fowl was given the untreated extract from 2 kilograms of polishings. It was entirely unaffected by this dose.

(*b*) A normal fowl was given the extract from 300 grams of polishings, treated with hydrochloric acid, and subsequently neutralized with sodium bicarbonate. The fowl was quickly prostrated and died within fifteen minutes.

(*c*) A fowl that had developed neuritis as a result of feeding on polished rice, and whose legs were completely paralyzed, was given untreated extract from 2 kilograms of polishings. No improvement.

(*d*) Another fowl that had developed neuritis, and whose legs were completely paralyzed, was given the extract treated with hydrochloric acid from 100 grams of polishings. This fowl was greatly improved and within twenty-four hours was able to walk like a normal bird; in fact, it was completely cured.

This experiment demonstrated that the difference in therapeutic action between Funk's extract and our own was undoubtedly due to the hydrochloric acid used in one case and not in the other, and, further, that the properties of our extract could be changed so that it resembled the action of Funk's extract by simple treatment with 5 per cent hydrochloric acid.

It was found that 5 per cent sulphuric acid effected the same transformation. It is apparent that strong mineral acids break up the protective substance which is present in the rice polishings and the alcoholic extract, reducing it to a more active and probably simpler form. This may be regarded as a hydrolysis which, however, is not produced by the weak organic acids normally present in the extract. Funk's(6) later results in working with yeast amply confirm our observations as to the effect of hydrolysis with strong mineral acids.

Having this clue, we again prepared an extract of rice polishings according to our previous method, hydrolyzed it with 5 per cent sulphuric acid, and precipitated it with phosphotungstic acid and silver nitrate and baryta, following Funk's procedure closely. We thus obtained a small quantity of a crystalline base, 30 milligrams of which promptly cured fowls suffering from polyneuritis. This base was undoubtedly the same as that isolated by Funk. The next step was to try a feeding experiment with this base.

*Experiment 44.*—A quantity of this base was dissolved in distilled water in such proportion that each 10 cubic centimeters of the solution represented the amount of the base extracted from 10 grams of polishings.

Four fowls were fed on polished rice, and were given a daily dose of 10 cubic centimeters of this solution. One fowl developed neuritis in thirty-five days, one in thirty-seven days, and one in forty-five days, when the experiment was discontinued. It appears from this experiment that the fowls may have received some protection, since the incubation period was somewhat prolonged. (Average incubation period of our experiments twenty-six days.) The protection afforded was but slight.

The explanation of the failure completely to protect these fowls undoubtedly lies in the fact that sufficient amounts of this base were not used. In the curative experiments relatively large quantities of this base were used; that is, 30 milligrams. Funk obtained only 0.4 gram of this base from 50 kilograms of polishings, and the amounts we have obtained from several hundred kilograms of polishings have been proportionately even smaller. It is, therefore, apparent that to cure one fowl a quantity of the base extracted from about 5 kilograms of polishings was used, or almost five times the total quantity used to feed 4 fowls. But if this base were the only protective substance contained in the rice polishings, and were completely extracted, it is evident that the equivalent of 10 grams of polishings should have fully protected. Since this quantity did not protect completely in a feeding experiment, we now considered the following possibilities:

1. That there are other protective, if not curative, substances in the polishings besides Funk's base.
2. That Funk's base is incompletely extracted by Funk's method.
3. That both of the above hypotheses are correct.

*Experiment 45.*—An extract of rice polishings was prepared according to our usual method and *without hydrolysis* was precipitated by phosphotungstic acid. The washed precipitate was then mixed with distilled water in such proportion that 10 cubic centimeters of the suspension represented the substances precipitated by phosphotungstic acid from 10 grams of polishings.

Four fowls were then fed on polished rice, and were given a daily dose of 10 cubic centimeters of this suspension. One fowl died of avian diphtheria after fifty-one days, and the other 3 birds remained in perfect health for seventy-one days, when the experiment was discontinued. This experiment showed that the total phosphotungstates precipitated from an unhydrolyzed extract of rice polishings were sufficient to prevent polyneuritis in a dosage corresponding to 10 grams of polishings, and even when fed as phosphotungstates, without any further treatment.

*Experiment 46.*—The hydrolyzed extract obtained from 10 kilograms of polishings was completely precipitated by phosphotungstic acid. The precipitate so obtained was extracted by repeated and prolonged shaking with 50 per cent alcohol. The phosphotungstic acid was then removed from this alcoholic solution of the phosphotungstates by barium hydroxide.[3] A sufficient quantity of this alcoholic extract of phosphotungstates was reserved to feed 4 fowls (group *a*). The remainder of the solution was carefully neutralized with sulphuric acid, and silver nitrate added until a drop of the clear solution gave a brown precipitate of silver oxide with cold barium hydroxide. The flocculent precipitate so obtained was filtered off and the silver removed by hydrogen sulphide. The filtrate from the silver sulphide so obtained was used to feed 4 fowls (group *b*).

The filtrate obtained from this first precipitation with silver nitrate was then rendered distinctly alkaline with baryta. The precipitate so produced was filtered off and the silver removed by hydrogen sulphide, and the filtrate from the silver sulphide was used to feed 4 fowls (group *c*). The filtrate remaining after these two precipitations by silver nitrate was used to feed 4 fowls (group *d*).

Sixteen fowls were fed on polished rice, and each group of 4 received one of these solutions in a daily dose equivalent to 30 grams of polishings, as follows:

Group *a:* Four fowls receiving alcoholic extract of total phosphotungstates. All 4 fowls remained well for sixty days.

Group *b:* Four fowls receiving silver nitrate precipitate in neutral solution (purine bases). One fowl developed neuritis in thirty-six days and died. A second fowl developed neuritis in forty-six days. A large dose of the solution was given this fowl and the remaining 2 birds. The sick fowl improved in health and ended the experiment on the sixtieth day almost entirely cured, and the other 2 birds remained in health for the full period of sixty days.

Group *c:* Four fowls receiving silver nitrate precipitate in alkaline solution (Funk's base). All 4 fowls remained well for sixty days.

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[3] This method was adopted, because in this way the phosphotungstic acid can be removed without subjecting the solution to a great excess of baryta, as must necessarily be done when the dried precipitate is broken up by barium hydroxide. The use of strong alkaline solutions is to be avoided, as they undoubtedly decompose a part of the protective substances.

Group *d:* Four fowls receiving filtrate remaining from the above double precipitation. All 4 fowls remained well for sixty days.

The experiment was discontinued after sixty days because the solutions prepared were exhausted.

Group *a* in this experiment simply served as a control, showing that the alcoholic solution of the total phosphotungstates sufficed to confer protection in the amount used. But groups *b*, *c*, and *d* all contained substances that differed from each other chemically. Group *c*, which contained Funk's base, might be supposed to confer protection, as it did; but group *b*, containing purine bases which have in the past been supposed to be devoid of protective action, and group *d*, presumably containing choline and other similar bases, also both conferred protection. The protection conferred in group *b* was not complete, it is true; but we have reason to believe, from the fact that the second bird to be affected by neuritis improved when larger doses were given, that the amount of protection is simply a question of the size of the dose of these purines given. If these purines had been fed to these fowls in large amounts from the first, complete protection might have been secured.

It is important to note that, of these three groups of substances which conferred protection, only one was promptly curative; namely, group *c*, which contained Funk's base. The substances in the other two groups were tried repeatedly on fowls suffering from polyneuritis and absolutely failed to produce prompt recovery from the paralyses, although the lives of the birds appeared to be prolonged by this treatment.

Therefore, we have evidence that there is in our extract of rice polishings:

(1) A substance (Funk's base) which, used in sufficient doses, will both protect fowls from developing polyneuritis and promptly cure fowls that have already developed the disease.

(2) Two other groups of substances which will protect fowls from developing polyneuritis, but which are incapable of promptly curing fowls already suffering from the disease. The latter groups of substances, therefore, have entirely escaped previous discovery, because all the other investigators who have so far attempted to isolate these vitamines have relied exclusively upon curative experiments. Therefore, it appears certain that there are several groups of chemical substances that are capable of protecting fowls against polyneuritis gallinarum. Unfortunately we are unable at this time to state positively that these substances do not contain Funk's base. For it is

possible, of course, that these different chemical compounds may all contain Funk's base as a nucleus. This question can be solved only by obtaining complete information as to the constitution both of Funk's base and of these several compounds. We had hoped to begin this work, but have been greatly hampered owing to the extremely minute amounts of Funk's base which so far we have been able to obtain. This leads us naturally to the consideration of the second point outlined above; namely, is Funk's base completely recovered by Funk's method?

From what has already been said with regard to the action of alkalies on the protective substances, it appears probable that Funk's base is not completely recovered by his method, but that the greater part of it is destroyed when the precipitated phosphotungstates are broken up by barium hydroxide, which is a powerful alkaline reagent. This seems the more probable from several observations that we have made. In the first place, the curative action of the simple hydrolyzed extract is very powerful, the extract from 200 grams of polishings being usually sufficient to produce a complete cure. It is evident from experiment 46 that precipitation with phosphotungstic acid does not injure the protective substance; but, when the phosphotungstates are broken up by barium and the decomposed product used for curative experiments, it is found that a quantity from twenty-five to fifty times greater than the original 200 grams of polishings is required to cure a fowl. Manifestly the greater part of the curative substance is lost in the manipulation, and it can only be the result of the use of the alkaline barium hydroxide. In the second place, during this decomposition of the phosphotungstates by barium a strong methylamine smell is given off as was previously noticed by Funk. In our experiments we have observed that the loss of curative properties appears to be proportional to the strength of this methylamine odor given off, and we deduce from this fact that the methylamine odor indicates the amount of decomposition of nitrogenous bases to which class the protective substances belong.

In the course of our attempts to discover a better method for the isolation of this base, we tried a method used by Suzuki, Shimamura, and Odake.(9) This method consisted in precipitating the unhydrolyzed extract by tannic acid, treating this precipitate with 3 per cent sulphuric acid, and subsequently removing the excess of sulphuric acid and tannic acid by barium hydroxide. By following this method exactly we succeeded in curing fowls by the extract so obtained, and therefore confirmed the above authors' observation. We also found that if the extract is hydrolized before precipitation with tannic acid, the curative substance is not then precipitated by the tannic acid, but remains in the filtrate. We did not succeed in isolating any greater amount of the curative base by this method than by Funk's method, doubtless because the former also depends upon the use of barium hydroxide to remove the tannic acid.

We had further hoped that the method described in experiment 46 might yield larger quantities of this curative base, since the excessive use of barium hydroxide in breaking up the precipitated phosphotungstates was thereby avoided; but after repeated attempts we were obliged to abandon this method also, because we found that the greater part of the curative base was lost during the final precipitation with silver nitrate in the presence of baryta. This loss may be caused by the action of the free barium hydroxide which is present during the precipitation, or may possibly be caused by the oxidizing action of the traces of free nitric acid remaining when the silver is removed by hydrogen sulphide, and the last traces of barium by sulphuric acid prior to evaporation. It appeared probable to us that larger yields could be obtained only by devising some method in which a prolonged treatment with barium hydroxide would not be employed.

In selecting more suitable precipitants, barium acetate was used for the following reasons. Funk believed that the curative substance is a pyrimidine base similar to uracil or thymine, and since pyrimidine bases are found only in nucleic acid it is, therefore, probably a constituent of nucleic acid. Several reactions which have been obtained with this base strongly confirm this view. Thus, the precipitation by silver nitrate in the presence of barium hydroxide, but not in neutral solution, places this base definitely in the pyrimidine rather than the purine group. The neutral reaction of the free base and its precipitation by phosphotungstic acid as a free base as well as in the unhydrolyzed form are in perfect agreement with this theory.

Now, if this base exists as a constituent of nucleic acid, it should be possible to obtain it from the basic barium nucleinate precipitated by barium acetate from the unhydrolyzed extract previously neutralized with barium hydroxide.

Accordingly the following experiment was performed:

*Experiment 47.*—A quantity of unhydrolyzed extract of rice polishings was carefully neutralized by barium hydroxide. An excess of barium acetate was then added and the precipitate so obtained was broken up or hydrolyzed by treating with 5 per cent sulphuric acid at 60° C. for three hours and filtered. The filtrate from this mixture, after the removal of the excess of sulphuric acid with barium carbonate, was used to treat fowls suffering from advanced polyneuritis, and we succeeded in promptly curing 3 fowls by administering to each quantities of this mixture corresponding to the extract from 200 grams of polishings. This is approximately the quantity of the original extract which, after hydrolysis, is required to cure such a fowl. From this it is clear that little or none of the curative power of the extract was lost during this process. This is a far better result than we have obtained by any other method we have used.

Moreover, this result is a strong confirmation of the belief that the curative base exists in food as a constituent of nucleic acid, and it may be well at this point further to consider this possibility. Schaumann(10) originally thought that the active principle of such foodstuffs as rice polishings, katjang idjo, etc., was probably nucleic acid, but he supposed that the active constituent of the nucleic acid consisted of phosphorus in some form. This has been shown to be incorrect. Grijns(11) in testing this hypothesis isolated the nucleins in an impure state from katjang idjo. His dried preparation contained 1.43 per cent of phosphorus pentoxide, and another preparation in watery suspension contained 3.2 per cent of phosphorus pentoxide. He was unable to protect fowls fed on polished rice by either of these two preparations. Grijns concluded that the nucleins did not contain the therapeutic principle of katjang idjo, a conclusion with which de Haan agreed in 1910. Schaumann obtained similar results; for in a later publication he stated that in the case of pigeons suffering from polyneuritis he could produce no effect by giving nucleins prepared from yeast. This would seem to be conclusive experimental evidence that the protective substance is not contained in nucleins. Chamberlain and Vedder in 1911(12) fed 4 fowls on polished rice plus 0.2 gram nuclein daily. They state:

> As 2 fowls out of 4 developed neuritis, it is not believed that the nuclein used had any decided power to prevent polyneuritis gallinarum. Since the incubation period for the 2 fowls which did develop neuritis is perhaps slightly above the average, and since 2 fowls remained well for fifty-six days, it can not be denied that there may have been a small amount of neuritis-preventing substance in the nuclein, a quantity sufficient to retard the onset of the disease.

Hoping that this result could be improved upon, we obtained a chemically pure nucleic acid from yeast (Merck's) and fed 4 fowls with polished rice plus large quantities (0.5 gram daily) of this nucleic acid. All 4 fowls developed polyneuritis within thirty days.

We believe that these confusing results are satisfactorily explained by the fact that the methods commonly used for the extraction of nucleic acid from proteid substances *depend upon the use of fixed alkalies.* Thus Neumann's(13) much-used method for the preparation of thymo-nucleic acid involves the use of 33 per cent sodium hydroxide, and Slade's(14) method for the preparation of yeast nucleic acid requires that the yeast be treated with 1.1 per cent of its weight of sodium hydroxide. Alkalies are also frequently used in the preparation of nucleins. We have already said enough concerning the loss of the curative base when treated with alkaline reagents to indicate that such methods would surely destroy it.

It seems probable to us, therefore, that the curative base exists in foodstuffs as a constituent of a nucleic acid, as is indicated by the chemical reactions of this base, but that it is not present in the nucleic acid or nucleins obtained from yeast or other substances by the use of alkalies. This accounts for the failures which have resulted so constantly when nucleins or nucleic acids were used as preventive or curative agents. The partial protection reported by Chamberlain and Vedder above may well have been due to the use of a nuclein in which the protective substances had not been completely destroyed, for the origin and method of preparation of this nuclein is unknown.

It is further known that the nucleic acid obtained from different sources differs in chemical composition. Thus the nucleic acid obtained from the thymus gland contains cytosin, thymine, and uracil, while the tritico-nucleic acid obtained from the wheat embryo contains uracil and cytosin, but not thymine. Therefore, it is very probable that this curative base exists as a constituent of certain nucleic acids, but does not occur in every nucleic acid.

The solution resulting from the acid hydrolysis of the barium acetate precipitate was much too complex to give crystals of the base by simple evaporation. Various methods have been tried to resolve this solution into its constituents, but so far without succeeding in isolating much larger quantities of Funk's base than had been obtained previously. Experiments in this direction are being continued.

We now wish to report certain experiments which we have performed in the treatment of human cases of beriberi. As a natural result of the great success obtained by the treatment of cases of infantile beriberi with the extract used by Chamberlain and Vedder(4) we proceeded to administer this extract to adult cases of beriberi.

*Case I.*—A woman who had been delivered about a month previously and was nursing her infant. She had been in good health until about a week before the time she consulted us, when her legs began to swell and became heavy. This became steadily worse and gradually extended to other parts of her body, and finally induced her to seek treatment. When first seen, œdema was pronounced and very general, having extended even to her face. Heart, lungs, and kidneys were apparently normal. A careful examination of the urine showed no albumin, casts, nor other abnormality. Her muscles were somewhat tender to the touch, and her reflexes were slightly impaired. She informed us that she had been living almost exclusively on polished rice. A diagnosis of beriberi was made and the case was kept under observation for a few days, during which the œdema became steadily worse, and finally the child developed symptoms of infantile beriberi, consisting of slight œdema and attacks of cardiac insufficiency. The case was now considered to be surely beriberi, and the patient was given the unhydrolyzed extract from 1 kilogram of rice polishings daily for three days. The œdema at once began to clear up, and had entirely disappeared by the end of the three days; the woman was able to walk better and the tenderness of the muscles was greatly relieved. The infant was not treated at this time.

*Case II.*—A woman in the Philippine General Hospital who was very kindly referred to us for treatment by Dr. A. G. Sison. This woman had been in the hospital for some time and was suffering from typical dry beriberi. Her muscles were much wasted and exquisitely tender, and she was completely bedridden. This woman was given the unhydrolyzed extract from 1 kilogram of polishings daily for two weeks with no improvement except possibly a slight alleviation of the tenderness of the muscles. She remained completely paralyzed. It may here be stated that we have been informed by several physicians that they have been similarly disappointed in the treatment of cases of dry beriberi with the extract of rice polishings.

*Case III.*—A man, who had suffered from dry beriberi for some time, was admitted to the Philippine General Hospital on September 6 suffering from an acute cardiac crisis. Through the courtesy of Doctor Sison we were invited to see this case and treat it. When first seen on the morning of September 9, the man was sitting up in bed gasping for breath, and the resident physician stated that he had been momentarily expecting his death during the preceding night. His pulse was 150, respiration 45, and his heart was palpitating violently. He presented the typical history and appearance of a case of chronic beriberi suffering from an acute cardiac exacerbation. The muscles of the entire body and limbs were wasted and extremely painful to touch, and in addition he had been unable to take any nourishment for several days because of persistent vomiting. The patient was at once (11 o'clock, the morning of September 9) given the unhydrolyzed extract obtained from 1 kilogram of rice polishings. This was retained, and his symptoms at once began to improve, and by the evening of the same day his pulse had dropped to 80, his respiration to 30, and the vomiting had ceased. On the following morning another acute exacerbation occurred, and he was again given the extract from 1 kilogram of polishings. Again the symptoms improved, and thereafter he was given the extract from 1 kilogram of polishings daily for about two weeks. The cardiac attacks never returned, the hyperæsthesia of the muscles was greatly relieved, the vomiting ceased, and his appetite returned; *but he still suffered from paralysis and was unable to walk.*

About one month was now allowed to elapse, during which time the condition of this man remained practically the same. He had had no recurrence of cardiac attacks, but he was still unable to walk. He was then given Funk's base, which we had extracted by Funk's method, from 10 kilograms of polishings. The crystalline base was dissolved in a small quantity of water for purposes of administration. The next day this man showed marked improvement, and for the first time since he had been in the hospital he made attempts to hobble around. This dose was repeated on the next day, and was followed by still further improvement, so that in a few days the man was able to walk about by holding on to the bed, chairs, etc. He was not completely cured, in the sense of being able to walk easily without assistance, but this we attribute to the fact that his muscles were so completely atrophied that they did not have sufficient power to support him. A similar result is seen after any disease which necessitates a long period in bed. We believe that the paralysis in this case was greatly relieved, if not cured, by the administration of Funk's base.

We were unable to treat other cases with this base owing to the minute quantities at our disposal.

It will be seen from these cases that the administration of extract of rice polishings, prepared according to our usual method, is capable of dissipating the dropsy in cases of wet beriberi, and of promptly relieving the attacks of cardiac insufficiency, but that this extract is incapable of curing the paralysis in cases of so-called dry beriberi. On the other hand, Funk's base isolated from this extract after hydrolysis is capable of promptly relieving the paralysis in dry beriberi. This closely parallels the results obtained in treating fowls suffering with polyneuritis. Œdema is not present in these fowls, and the disease is apparently similar to human dry beriberi. The untreated extract when given to these fowls fails to cure promptly the paralysis, but Funk's base obtained from this same extract will promptly cure the paralysis.

The exact relationship existing between dry beriberi and wet beriberi has never been satisfactorily explained. For many years clinicians considered them to be distinct diseases, but since it has been found that cases of dry beriberi sometimes develop œdema or become "wet," while cases of wet beriberi sometimes lose this œdema, and thereby become "dry," it has been generally accepted by clinicians of the present day that the two conditions are manifestations of the same disease, beriberi. However, this belief has never been definitely proved, and we are still in the dark as to why some cases of beriberi should be "dry" and some "wet." In a previous paper(15) the hypothesis was suggested that rice polishings and other foods contain two substances or vitamines that are essential for proper metabolism, one of these being a neuritis-preventing vitamine, and the other a substance which affects metabolism in such a way that its absence results in œdema and cardiac failure. It is believed that the results just detailed in the treatment of cases of beriberi afford some support for this hypothesis. Thus it is clear that, since cases of wet beriberi and cardiac beriberi may be promptly relieved by the administration of the untreated extract, while cases of dry beriberi receive little or no benefit from its use, the extract must act differently in these different classes of cases. It seems probable that this different effect may be due to the fact that there are two different chemical substances or vitamines in the extract, one of which acts directly in cases of wet beriberi, while the other occurs as a constituent of some substance (nucleic acid) in such chemical form that it is only available for immediate curative effects when it has been broken up.

Realizing that these results, while suggestive, are not complete proof of this hypothesis, experiments were planned to afford more satisfactory evidence. Thus we intended to treat cases of wet beriberi with Funk's base. If our hypothesis that there are two vitamines is correct, the œdema should not be relieved by this treatment, since Funk's base has been shown to be the neuritis-preventing and curing vitamine.

We were unable to try this experiment, because we have been unable to obtain cases of wet beriberi in Manila at this time.

It is known that the neuritis-preventing and curative vitamine (Funk's base) is completely precipitated by phosphotungstic acid. It seemed that, if the vitamine of wet beriberi actually exists as a separate chemical substance, it might be possible to effect a separation by this method, if it should happen not to be precipitated by phosphotungstic acid. Accordingly, we took a portion of the extract of rice polishings which had been used in the treatment of the above cases and precipitated it with phosphotungstic acid. This precipitate was filtered off and the filtrate used to treat a case of infantile beriberi. Infantile beriberi belongs most frequently to the wet or cardiac types of the disease. This child suffered from œdema and from attacks of cardiac insufficiency. The child was promptly relieved as the result of this treatment, the œdema disappeared, and the heart returned to a normal condition, although it still continued to nurse its beriberic mother. This case is a strong confirmation of our hypothesis.

We realize fully that we cannot claim to have proved this hypothesis upon the basis of such a small number of cases; but, as we are at present compelled to discontinue this work, the facts so far obtained are reported in order that others may continue these experiments and obtain a definite proof or disproof of this hypothesis, which we are led to believe is the correct explanation of the difference between wet and dry beriberi.

## CONCLUSIONS

1. Undermilled rice may be stored for one year in a damp place without losing its protective powers against polyneuritis gallinarum. It is improbable therefore that a rice which originally affords protection against beriberi will lose this property by storage even in damp places.

2. The neuritis-preventing substances or vitamines contained in rice polishings are only slightly soluble in cold 95 per cent alcohol, since three successive extractions, using a total of 6 liters of alcohol to each kilogram of polishings, fail to remove all of the neuritis-preventing substances from rice polishings.

3. Strongly alkaline reagents, such as sodium hydroxide, ammonia, and barium hydroxide, destroy the neuritis-preventing vitamine in its free or unhydrolyzed state, and the use of these reagents must be avoided in endeavoring to isolate this substance.

4. Basic lead acetate does not precipitate the neuritis-preventing vitamine, and a considerable portion of this substance may be recovered from the filtrate.

5. The therapeutic properties of an alcoholic extract of rice polishings are greatly altered by hydrolysis (treatment with 5 per cent hydrochloric or sulphuric acid). The unhydrolyzed extract is not poisonous and is only slowly curative. The hydrolyzed extract is exceedingly poisonous in large doses and promptly curative in small doses.

6. We have confirmed Funk's observations by isolating a crystalline base from an extract of rice polishings by Funk's method. This base in doses of 30 milligrams promptly cured fowls suffering from polyneuritis gallinarum.

7. Funk's base or vitamine is present in rice polishings in considerable amounts, and only a very small portion of it can be obtained by Funk's method.

(1) Because the polishings themselves are incompletely extracted.

(2) The greater part of this base is lost during the chemical manipulations required by Funk's method as shown by the facts:

(*a*) The curative action of this base, isolated, is from twenty-five to fifty times weaker than the curative action of the original hydrolyzed extract.

(*b*) When fowls are fed on polished rice and given a daily dose of this base in amounts corresponding to 10 cubic centimeters of the original extract, these fowls are not protected. Ten cubic centimeters of the original extract or 10 grams of polishings daily are amply sufficient fully to protect fowls.

(3) Because Funk's method depends upon the use of barium hydroxide, and we have shown that this reagent destroys this base.

8. Two groups of substances (purine bases, choline-like bases) may be isolated from rice polishings in addition to Funk's base and are capable of partly or wholly protecting fowls fed on polished rice against polyneuritis gallinarum, but are incapable of curing fowls that have already developed the disease. The chemical nature of these two groups of bases requires further investigation.

9. We have confirmed the observation of Suzuki, Shimamura, and Odake, that Funk's base may be precipitated from unhydrolyzed extract by tannic acid, but did not succeed in obtaining large amounts of this substance by this method.

10. It is probable that this base or vitamine exists in food as a pyrimidine base combined as a constituent of nucleic acid, but that it is not present in the nucleins or nucleic acids that have been isolated by processes involving the use of alkalies or heat.

11. The administration of unhydrolyzed extract of rice polishings to cases of adult wet beriberi, or to cases suffering from acute cardiac insufficiency, results in the prompt dissipation of œdema and relief of the cardiac symptoms.

12. The administration of unhydrolyzed extract of rice polishings to cases of dry beriberi is followed by little or no improvement in the paralytic symptoms.

13. The administration of Funk's base to cases of dry beriberi is followed by an immediate improvement in the paralytic symptoms. This should remove the last doubt that dry beriberi is caused by the deficiency of this substance in the diet. It also finally proves that dry beriberi of man and polyneuritis gallinarum are essentially the same disease.

14. We have succeeded in curing a case of infantile beriberi (of the wet type) by administering that portion of the extract of rice polishings represented by the filtrate from the phosphotungstic precipitate. Since this filtrate does not contain Funk's base, this is evidence that wet beriberi is cured by some other substance.

15. Conclusions 11, 12, 13, and 14 are striking confirmatory evidence for the hypothesis previously stated by Vedder and Clark that wet beriberi and dry beriberi are two distinct conditions, each being caused by the deficiency of a separate vitamine.

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# THE BIOLOGY OF TABANUS STRIATUS FABRICUS, THE HORSEFLY OF THE PHILIPPINES

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Seven plates

The importance of investigating the bionomics of insects capable of transmitting disease need not be emphasized. However, the paucity of literature on this subject leads one to suspect that insects as pathogenetic factors are not sufficiently appreciated by the entomologist. Too often this negligence of the entomologist has to be corrected by the overburdened research worker in medicine, in order to supply important links in the etiology and transmission of infectious diseases.

*Tabanus striatus* Fabricus[2] is the most prevalent horsefly of the Philippines. It has a possible economic bearing relative to trypanosomiasis, in as much as it is found wherever surra is abundant, and the seasonal distribution of the fly and the disease are in a measure coincident throughout the principal regions of the Archipelago. In Manila and within a radius of 50 kilometers this *Tabanus* is found rather continuously from October to March. It is during this period that surra outbreaks in this region are the most prevalent. It is during this period also that other species of bloodsucking flies have their seasonal preponderance. Species of *Tabanus* have been experimentally incriminated in the transmission of surra in India, in North Africa, and elsewhere. In the Philippines *Tabanus* has never been proved to be a carrier of trypanosomiasis. At the present time this fly is the subject of an experimental investigation to determine its relation to the spread of surra. With this purpose in view, it was found essential first to breed the fly preparatory to use in transmission experiments, and the data collected and presented here are the results of the rearing of thousands of flies under laboratory conditions in the veterinary research laboratory at Alabang, Rizal.

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[2] I am indebted to Professor James S. Hine of Ohio State University and to Mr. Austin of the British Museum for this identification.

The writer had been unsuccessful in locating the eggs or larvæ in their natural habitats.[3] Special efforts were made during three months, prior to the appearance of the imago, to find the young forms by searching plants and stones and by using a water net along the course of the neighboring creek and along the shores of the lake within a kilometer of the laboratory. Except for the absence of this material, the local conditions were ideal for the complete study of the bionomics of this species of fly. At the foot of the hill upon which the research laboratory is located, a lane of rain trees (*Enterolobium saman* Prain) divides a pasture land from a low, wet area which drains into the adjoining creek. The pasture land was used by a herd of about 50 cattle which passed back and forth along one side of the rain trees. The *Tabanus* when present used the cattle for feeding and the rain trees adjoining for resting when engorged or to escape from the sun's heat. During the early part of the day, probably until 2 o'clock, the cattle were disturbed by the attacks of many of these gadflies, which during the hottest portion of the day and through the night rested on the trunks of the convenient rain trees. Had the creek served as a harborage for the eggs and young forms of the fly, the ecological relations for a biological study of this fly would have been complete.

To obtain the eggs for study it was found necessary to keep the flies in captivity in a structure built for this purpose. This consisted of a capacious fly-proof building with brass-gauze sides and top and cement flooring, sufficiently large for from 4 to 6 big animals. One section was provided with a concrete water tank and growing plants. Here 2 carabaos were placed with thousands of flies, which were gathered while resting on trees along the road. In a short time females were observed feeding on the hosts provided, and several were found ovipositing in various places about the inclosure.

## OVIPOSITION

The time selected for egg laying under the conditions provided was invariably during the early afternoon, never later than 2 o'clock. This was observed in nearly 50 instances.

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[3] Subsequent to the writing of this paper, larvæ and pupæ have been discovered in large numbers buried in sand at many points on the shore of Laguna de Bay.

The eggs are laid in a compact mass either extended on a flat surface or surrounding various attached objects, usually of small diameter, such as projecting splinters of wood, suspended fibers of jute sacking, fine brass wire, a single animal hair, and coarse iron wire. Upon these materials the eggs are laid in an ellipsoidal form sometimes surrounding the objects completely or nearly so. On one occasion 2 egg masses were found upon a small splinter of wood which they entirely enveloped. The surfaces of the egg masses were continuous, so that the double mass resembled a single large one. When eggs were found deposited on a flat surface, on two occasions a leaf was the object selected. These were leaves of an ornamental plant which was used for shade purposes in the breeding cage. The plant in question grew close to the cement water tank in the breeding cage. In all other instances the eggs were deposited upon woodwork on the sides and ceiling of the cage, invariably upon the shaded portions, as the underside of beams and partitions. In egg laying upon flat surfaces there was a strikingly constant geometric form. Usually the form assumed was roughly a pentagon with a biconvex center.

At the beginning of oviposition usually 2 eggs are deposited in the position of an inverted V. Three to 4 eggs are then laid on either side of the apex of this V, and then one side and then the other is built up, rather irregularly at first, until the sides of the pentagon are completed. The eggs are laid cleanly and definitely, each line slightly overlapping that preceding. When the eggs are laid in the extended order, they are deposited 3 or 4 layers in depth, but usually as many as 6 layers are required to complete the mass when the eggs surround a convex object.

In the process of laying, the body is held away from the egg mass, the legs being planted firmly. When the eggs are attached to an object above, the insect stands with head downward, the forelegs suspended alongside the head, the hind and middle legs supporting the weight of the body. At the first movement, the anal end of the body is bent toward the thorax under the abdomen, and with a slight jerk the egg is laid, while the brush-like appendage of the ovipositor exudes a tiny drop of liquid coating the egg as it is deposited. The movement of deposition is very much like squeezing a bit of pasty material from a collapsible metal tube.

In several counts that were made, the fly was observed to lay with clock-like precision at the rate of 10 eggs per minute. This did not vary, whether the attached object was above or below the fly. In three instances observed, the process occupied from forty to forty-five minutes. Both the beginning and completion of the performance were without deliberation, the insect walking away from the mass of eggs and flying off as soon as the last egg was deposited.

When disturbed during oviposition, the insect does not fly and can readily be carried without attempting to escape. While in the act of laying, if interrupted and dislodged from the position, it immediately begins to deposit a new egg mass. This was twice repeated with one female, and three distinct egg masses were deposited, all of them identical in geometrical arrangement.

The eggs of this species of *Tabanus* are laid with very little cementing material. The cement used is a transparent substance and not dark and opaque as found to exist in the species described by Hine.(1) The cement provided by this species was tested and found to be waterproof, as well as insoluble in various grades of alcohol and xylene.

The eggs when laid are a pale clay yellow, but within twenty-four hours become slightly darkened with an ashy gray tinge. Microscopically fine black striations can be seen running lengthwise for nearly 0.5 millimeter from the end opposite the micropyle.

The shape of the individual egg is that of the muscid type with more sharply pointed ends; it is not quite spindle-shaped. Several eggs were measured and found to average in size 1.6 by 0.4 millimeters. The size of the mass varies from 9 to 12 millimeters in length by 6 to 9 millimeters in breadth.

The number of eggs laid in a mass varied greatly. In 4 masses counted, there were respectively 270, 340, 417, and 425. Ten masses dissected from the bodies of killed flies were found to average 405; the greatest number found in any female was 495.

## THE HATCHING PROCESS

Two egg masses were observed microscopically during the entire process of hatching, and 14 egg masses were noted as to the length of the incubation period. The minimum period observed was three days and the maximum five days. Four days is probably the average length of time required for incubation. It was observed that the degree of temperature and moisture influenced the time of hatching. Slight changes in either of these factors can be used to control the time of emergence from the egg. The following is a series of observations on the hatching of an egg mass.

Twenty-two hours previous to the hatching of the embryo, certain unmistakable activities were discernible in the egg. The first signs of these were seen in the two eggs which formed the nucleus for the egg mass and which are the first eggs laid. These movements, as indicated by either of the dark eye spots, could be seen with a hand lens at intervals of a few seconds; their action was similar to that of the bubble in a spirit level. In about an hour the movement was seen to be rather general in the egg mass, accompanied in the eggs first laid by an alternate collapsing and distending of the exochorion. This action is the result of the torpedo-like movement of the head capsule of the embryo in the direction of the micropyle of the egg. The movement is effected by the piston-like action of the apophyses of the cephalopharynx, which appear to work alternately, bringing the saw-toothed mandibles in contact with the micropyle canal. These movements proceeded uninterruptedly during the hours of the night, the only change observable being that the body segments of the embryonic larva became better defined. At 4.25 the next morning the segments of the embryo could easily be counted through the chorion. The dorsal surface of the exochorion was seen to be slightly shrivelled.

Fully one hour and thirty minutes intervened during which there was no action worth noting. This quiescence was interrupted by a sudden remarkable activity of all of the visible eggs of the mass. At 6.08 there was a general upheaval of the surface of the egg mass, an agitation within the eggs, and an alternate collapsing and distending of the eggshells. At 6.10 the first layer of eggs gave birth to a silvery horde of young larvæ, which at 6.12 had crawled from view. Then ensued another spasmodic agitation giving birth to another lot of larvæ, which crawled from the mass of empty eggshells. The emergence which is effected by the head structures is aided by the posterior protuberances, which functioning as prolegs push the body of the larva clear of the eggshells.

## MORPHOLOGY AND HABITS OF THE YOUNG LARVÆ

Immediately after emerging from the egg, the young larvæ seek concealment. In nature, no doubt, resort would be had to the convenient water course where aquatic plants, drift wood, and stones would be the probable hiding places. The larvæ under observation became very active and crawled out of the stender dish, a height of 9 centimeters, and tumbled into the water of the basin provided. When collected and placed in a deep glass vessel with some water, the entire mass took refuge behind the filter paper in the glass. Here they crowded side to side with their siphons projecting from the upper edge of the paper. When disturbed and forced to take to the water, they were found in thirty minutes reassembled in the characteristic gregarious fashion behind the filter paper against the glass.

For convenience in study, a majority of the larvæ were transferred when 1 day old to individual glass jars one-third filled with clean wet sand from the lake shore and provided with strips of filter paper soaked in muck from the creek bottom. The jars, which were the common half-pint jelly glasses recommended by Hine, were kept covered with filter paper, held in place by the tin lid which had a disk cut from its top to admit air. By renewing the moisture on the strip of filter paper in the jar, the filter-paper cover serves ideally to control the humidity.

A considerable number of the larvæ were not separated, but were left together for observation in a glass dish with a few strips of paper saturated with muck from the creek.

The larva one hour after hatching is 1.5 millimeters in length. The following day several were found to measure 1.8 millimeters. The general color is a dirty white with a tracheal system of waxy white, the abdominal contents pale green, and the Malpighian tubules of a lilac color. There are 2 black eye-spots located midway on the head capsule. The latter tapers to a sharp-pointed mouth with a prominent pair of great hooks or mandibles. The segments are provided with typical, conical, truncated prolegs, each armed with a chaplet of medium long, brown hairs. The siphon which is carrot-shaped at this stage is a prominent feature.

Food in a variety of forms was furnished the larvæ. They thrived from the start on minute crustacea, larvæ of *Stomoxys*, mosquito larvæ, and young angleworms. Full-grown angleworms were found unsuitable, and larvæ of the blowfly and flesh fly were not satisfactory unless killed previously, as they were capable of killing or injuring even well-developed *Tabanus* larvæ. As soon as the insect becomes aware of the presence of food, the claw-like mandibles are protruded from the head capsule, and bury themselves in the live food like meat hooks. With a slight curve dorsally, the larva's body is brought forward, and a small portion of the food is lacerated. This is aided by a twisting of the head and a pulling with the extended jaws. The mandibles are brought together with a rapid clawing action, the parts working in apposition. When prehension is effected, the jaws move alternately upward and downward and laterally, and the bolus is swallowed in fibrous strands.

Seeking and devouring food is not a continuous operation as it is in the case with *Stomoxys* and the dung flies. The *Tabanus* larva requires a long rest after a sufficient meal is taken. A 2-day-old *Tabanus* is capable of devouring 2 half-grown larvæ of *Stomoxys* in twenty-five minutes. In one instance a full-grown *Stomoxys* larva was destroyed in exactly twenty minutes. Here the attack on the *Stomoxys* was made through accidental collision, the *Tabanus* instinctively thrusting out its mouth and tentatively taking a bite. It apparently became greatly excited (this was its first meal), and, thrusting its head into the body of the *Stomoxys* larva, commenced to probe by twisting its head rapidly. In less than a minute the cuticle was broken through and an ample slit was made through which the entire head was buried in the body of the victim, whereupon an energetic gouging took place. The *Tabanus* worked through the cephalic third of the body upward to the head, then worked in the other direction on the lower two-thirds. This gouging was continued until the *Stomoxys* had become completely eviscerated, during which time the head of the *Tabanus* kept steadily probing, twisting its pharynx from side to side, and pushing forward with its rostrum until the *Stomoxys* larva was completely devoured with the exception of the cuticle.

The full-grown *Tabanus* larva does not wait for its food, as is the tendency in the young stage, but actively pursues its prey. When an angleworm is seen, perhaps 2 millimeters distant, the elastic head capsule of the larva darts forth, curves its claw-like hook about the worm's body, and, with its head curled under its struggling prey, retreats quickly into the sand until all but its cephalic end is hidden. It begins to feed then, devouring in twenty minutes an angleworm fully four times its own length.

The intestinal tract seen through the hyaline cuticle soon partakes of the color of the food ingested. The color is pale brown when the food consists of the wet muck in which crustacea and minute forms are sought. As a result of feeding on blowfly larvæ and angleworms, the young *Tabanus* assumes a variegated coloration. The intestinal tract then appears tinted with green, yellow, brown, and red particles of the food.

In one set of larvæ the origin of cannibalism as an acquired habit was observed. This was seen in larvæ which had been kept together for four days since their birth. Until that day no food was offered them except that which they might have obtained from the surrounding creek water. Apparently they lived together amicably with their bodies compressed against the glass dish and the bit of filter paper. A live angleworm was placed in the glass dish while the resting larvæ were observed with a lens. The worm was not placed in the immediate vicinity of the mass of larvæ, but nearly 4 centimeters distant. The presence of the food appeared to act as a stimulus. No movement was made toward the worm, but each larva appeared to become greatly excited and began to prod the larva nearest to it and to nip its neighbor's appendages; several very marked instances of laceration were noted. This doubtlessly marked the beginning of systematic cannibalism. From this cause, 39 of the 265 larvæ kept in a large glass dish were destroyed within four days. Four dead bodies were recovered. Upon another occasion the extent of cannibalism was very much more marked. A lot of 415 larvæ which hatched on November 12, 1912, was placed in a deep glass dish with moist lake-beach sand, and fed daily on angleworms. Each morning it was observed that only about one-half of the worms supplied the previous day was eaten, so that with the daily fresh supply more than enough food was present. Another lot of 300 larvæ, the same age as the preceding, was kept in individual glasses under similar conditions. On December 6, counts were made of the survivors in the large glass dish. Thirty-five larvæ remained, of which 18 were the maximum size, 11 were a little more than one-half this size but equal to the largest found in the individual jars, and the remaining 6 larvæ were so small as to be easily overlooked. The census taken of the larvæ from the individual jars showed a loss of 12, or less than 5 per cent. Allowing 5 per cent for loss from other causes, it appeared that above 85 per cent of the larvæ kept together in the large jar was destroyed through cannibalism.

It has been observed by Hine in other species that a *Tabanus* larva is enabled to survive for a few days in the absence of food. In this species likewise there seems to be a decided resistance to starvation, two instances showing periods of ten and twelve days.

The movements of the body are in general similar to those of larvæ of the muscid type. There is a general progressive peristaltic movement, invariably accompanied by a decided telescoping of the segments. The head is raised as the prolegs of the anal end push the body forward, then it is lowered. The mouth is projected when the head capsule is extended, but recedes quickly when the glass sides of the container or any obstacle is encountered. The larva can easily move backward for a considerable distance. This it does if wedged in a tight place or in capturing food when it retreats into a channel previously made in the sand.

The larvæ readily adapt themselves to a watery medium. They can remain submerged for several minutes at a time without apparent discomfort. When placed in deep water the movements of the body are a general struggling without apparent definite purpose. At any rate, there is little or no progression, the body doubles like a bow, the head and tail meeting, then straightens with a whipping action. In swimming, the body is held along the surface of the water and the siphon is extended toward the air in a manner very suggestive of the larva of an anopheline mosquito. The principal movement observed is that of simple telescoping of one segment into another. When speed is required or an obstruction is to be passed, there is a vigorous whipping movement of the siphon laterally, toward and away from the head. This latter movement is also noted when the insect is disturbed.

When a young larva is placed in water containing entomostracans or other minute animals, a barely perceptible churning of the liquid occurs in the region of the mouth. This disturbance is no doubt caused by the movements of minute tentacles which assist in procuring food. These tentacles form the armature of the stomal disk, consisting of a process arranged like a turnstile mounted on a pitted chitinous plate at the base of the great hook or mandible. In the very young larva the stomal disk appears as a chaplet of delicate chitinous rods. When a larva is treated with strong caustic potash, the stomal disk appears to be the only structure which resists its action, the other chitinous structures, even the heavy pharyngeal apophyses, are bleached. In common with the other chitinous portions of the head capsule the stomal disk is shed at each of the three ecdyses.

## GENERAL DEVELOPMENT OF THE LARVA

The young larva shows in its form and behavior its adaptibility to an aquatic life. This is well illustrated when a larva is turned adrift in an aquarium containing mosquito wrigglers. The *Tabanus* has no difficulty in keeping afloat with them and foraging at will upon the active culicid larvæ. *Tabanus* larvæ have been observed capturing wrigglers, holding them by their jaws under the water, and actually killing the culicid through drowning. In one instance a *Tabanus* larva held its victim, which was fully five times its size, suspended beneath it in such a manner that the culicid was unable to project its siphon for breathing purposes, while that of the *Tabanus* was functional. The *Tabanus*, obtaining a secure perch by dragging itself and the prey above the water, devoured the mosquito wriggler in a few minutes. In another instance the weight of the culicid pulled its captor under the water to the sandy bottom a distance of nearly 30 centimeters. Here the *Tabanus* showed its superior vitality by remaining attached for nearly two minutes until apparently assured of the immobility of its prey, then, releasing its hold, the *Tabanus* larva struggled to the surface where it rested with siphon extended. The mosquito larva meanwhile moved feebly several times, and succumbed within a few minutes.

This adaptability is lost, however, in the developed larva which becomes more slothful in movement and grub-like in superficial appearance. Both extremities, the head and the siphon, become obtuse in form, and the ventral protuberances functioning as prolegs become more truncated. Growth after the second molt becomes noticeably less in length and more in thickness. The greatest growth observable was shown to be between the periods of the first and the second molts.

The following table is given to show the normal growth of a larva. The measurements and the critical stages of life are indicated:

TABLE I.—*Progress of development of a larva.*

| Date. | Length. | Stage of development. |
|---|---|---|
| | *mm.* | |
| Sept. 15 | 1.5 | At birth. |
| 16 | 1.8 | 1 day old. |
| 20 | 3.0 | |
| 21 | 4.0 | |
| 22 | 5.0 | |
| 23 | 6.5 | |
| 26 | 11.0 | After first molt. |
| 30 | 20.0 | |
| Oct. 8 | 22.0 | |
| 9 | 25.0 | Second molt. |
| 12 | 27.5 | |
| 17 | 29.5 | Mature larva. |
| 21 | 27.5 | |
| 24 | 17.5 | Pupa, third molt. |

In all biological accounts of the Tabanidæ there appears to be one phenomenon which is uniformly noted. This is the remarkable difference in growth shown by individual flies of the same species. The only process in the development which seems to be synchronous is the hatching of the eggs. After that the variations in time of development are extreme. In *Tabanus striatus*, for example, some larvæ twelve days old measured 3 millimeters, while others under precisely the same conditions measured fully 11 millimeters. In another instance 2 flies emerged as well-developed imagoes October 31, while 27 of the same brood still remained apparently healthy in the larval stage December 20.

### THE ECDYSES

In the very meager literature available I have been unable to find any reference to the molting process in Tabanidæ. It is referred to indirectly by King(2) at Khartoum, who found in *T. biguttatus* the shed larval skin adhering to the puparium.

The process of shedding the skin was observed in a great many instances. The time of molting of a brood of larvæ is extremely variable, which is consistent with the great variations noted in the time of development in general. The process has been accurately noted in two individual larvæ, although observed superficially in numerous others. The three molts are similar in their general aspects, the main distinction being the more profound changes produced in the insect at the later molts.

The usual preparations for molting were observed in this species. The premonitory signs were the refusal of food, uneasiness when exposed to light, desire to find a remote corner, and finally the stiffening of the cuticle. In one instance the larva was found in one spot pressed against the glass for three days. Here, between the sand and the glass of the jar, an excrementous cement was used to fasten the end of the abdomen. This material holds the end of the body very securely, although the remainder of the body requires free lateral movement. By the time the ecdysis is completed, the head has moved 3 millimeters from the spot where preparations for the process are made, while the anal end has retained its original position.

The shedding of the skin usually requires several hours; in one instance, due no doubt to interference on the part of the observer, the time was nearly twenty-four hours. In the first and second molts, splitting of the cuticle begins at the thorax, resulting in the tearing out of the entire head capsule which adheres to the molt during the remainder of the process. The anal segments are molted finally and the larva, emerging in its new skin, crawls its length on the cast skin and rests alongside it for two or more hours.

The first molt begins with larvæ 7 days old, the majority molting before the tenth day. The second molt usually occurs after an interval of at least four days, and in some larvæ as late as eight days, that is, when 15 to 18 days old. The time of the third molt precedes immediately the appearance of the puparium. This period, as has been noted, shows the greatest diversity among individuals of the brood. The third molt in 12 instances was shed between the ninth and twelfth days of life. In other individuals the process was not completed within three months, yet the adult fly was an apparently healthy insect.

Certain unimportant changes in morphology, dependent on the molting process, are noticeable. The loss in size due to contraction of the cuticle preparatory to ecdysis is usually compensated by a substantial extension immediately following the process. The extent of shriveling of the cuticle is represented by 1 millimeter in the first molting, 1.5 to 2 millimeters in the second stage, and 2 to 3 millimeters preparatory to the third stage. There is a notable increase in length resulting from the second ecdysis. A larva, measuring 22 millimeters on the day previous to the shedding of the skin, measured fully 25 millimeters the following day. In measurements of this sort one must make allowance for the extraordinary amount of telescoping of segments. As much as 5 millimeters may be involved in this process.

The structures mainly involved in the ecdysis are the tracheal system and the appendages of the head. The anal wing of the trachea constituting the siphon is drawn off in each molt in a perfectly cylindrical form. The body trachea is torn from its connections in irregular strands. The entire head capsule, including the chitinous pharyngeal framework, the great hook, and other mouth structures, are found in perfect form in the various exuviæ. These parts upon renewal in the larva become more heavily reinforced. The exuvia is usually in a good state of preservation; crumpled to be sure, but it can be extended in alcohol to three-fourths the length of the larva. Following each ecdysis, the larva is invariably leaden gray with tracheal strands of waxy white. Three anal segments including the siphon become lead colored and stiffened in structure. They are at this stage more truncated, with an anal band of cuticle 1 millimeter in depth, making the siphon appear somewhat atrophied. This is no doubt consistent with its restricted function. The color of the viscera has changed from the brilliant red and yellow to an indeterminate white, and the lilac tint of the Malpighian tubules has changed to a salmon color. These latter changes are due probably to a clearing process, in which the larva indulges during the somnus preceding each ecdysis.

After the second molt the fleshy protuberances functioning as prolegs become reinforced with a slight cuticular ring at their bases. The mouth parts at this stage are heavily chitinized. The great hooks or mandibles show a marked serration of the biting edge. The head projects more, exposing the dark brown ocelli, which prior to the second molt are seen only through the cuticle of the thorax situated nearly on the middle of the concealed head capsule.

The signs characteristic of the final molt are refusal of food, restlessness, attempted migration, and finally burial in the sand at the bottom of the jar. The body decreases slightly in length, but the thickness remains the same.

On the extremity of the abdomen tiny tubercles appear which project more from time to time, becoming tapering and spike-like. Near the caudal end of the abdominal segments, roots of hairs appear. These at first resemble brown spots of pigment and gradually lengthen into stiff brown hairs. The cuticle on the body becomes stiffened and shingle-like at the joints of the segments. The latter telescope less, and one can see numerous particles of sand embedded in the joints of the segments. These sand particles have been carried in during the telescopic movements of the abdomen.

After the fully developed larva passes through a period of semidormancy buried in the sand, the skin is seen to be ridged with cuticular plates. The head region is reinforced by stiffened cuticle, and the mouth orifice is closed by a plug of hard rose-colored cuticle. This pigmented material lines the entire pharyngeal sinus, plugging the mouth and the cephalopharynx. The cuticular plug has a substantial fold which forms a slit for the passage of the molting mouth. Caudally a similar impervious mass closes the opening of the siphon. A cuticular collar strengthens the base, and the connective tissue surrounding the trachea of the tract of the siphon tends to contract. Then the supports of the central trachea are gradually cast loose by a gentle wriggling of the insect's body. About this time there is a general wrinkling of the epidermis, the folds telescoping upon each other, and the surface becomes parchment-like.

Synchronous with the primary contraction of the segments, a light pea-green suffuses the last 3 segments of the body. The remainder of the larva changes to this color overnight. By morning the abdominal segments have changed from green to ocher, when the molting of the cuticle ensues. The shedding takes place in sections. The chitinous framework of the head is thrown off like a hood. This portion is everted upon the body, and remains dangling from the exuvia during the process. One-half the length of the skin is loosed on the side opposite to that to which the chitinous framework of the head is attached. This is shed by a peculiar auger-like movement of the tail end which is not attached to the glass or other object in the container, as in the previous molts. The skin is virtually unrolled from the detached head to the anal end, where it lies in a crumpled heap. Then the skin of the other side of the body begins to be shed. The chitinous framework constituting the former head capsule of the larva becomes rolled up in the exuvia, while the skin is torn slowly from the new membrane. When the first half of the skin is peeled off to the anal tip, the cast skin becomes attached to some object. In this instance the glass of the jar served as an anchorage during the remainder of the ecdysis.

The upper half of the body of the newly molted larva is encased as in an armor in pouches and pads of integument, outlining in a gauzy film the future appendages of the fly.

### GENERAL DESCRIPTION OF THE FULL-GROWN LARVA

The length is 28 to 29.5 millimeters; the width, 3 to 4 millimeters. The anterior half of the body is a greenish yellow, the remainder is a dirty white. At this stage the form is grub-like.

The head capsule, which occupies one-fifth the length of the larva, is a cylindrical bulb, formed by the invagination of the thoracic ectoderm. It supports the eyes, the antennæ, and the mouth parts. It is bound by a framework of chitinous rods, the cephalopharyngeal apophyses. This structure, observed through the thorax when the insect is in action, is composed of 4 black, medium-thick skeletal pieces running the length of the 3 cephalic segments in the form of a pyramid, with its apex provided with the external mouth parts. It terminates in the claw-like mandibles which are similar in color and texture.

The mandibles are heavy, powerful structures, slightly serrated on their inner surfaces. The musculature of these appendages permits the two elements working in apposition. At rest they are held horizontally, and can be projected suddenly and thrust vertically downward, which is obviously of great assistance in grasping the prey.

The palpi and antennæ in this species are silvery white, and usually found glistening with moisture.

The eyes are oval in shape, with the long axes parallel. When the larva is prepared to molt, the pigmented spots are usually distorted. In this species the eye spots or ocelli are very prominent, especially in the younger stages of the larva. They can be first seen in the embryo where they appear as dark beaded structures through the chorion of the well-developed egg. In the young stage of the larva the eyes appear in the pharyngeal cavity midway between the mouth and the cephalopharynx, and as growth continues they are located nearer the distal end of the head capsule; so that when the larva is full-grown and the mouth structures protrude in locomotion or prehension the eye spots are seen to project on the head capsule with the mouth parts.

The trachea which terminates in the conical tubular siphon is lead gray in contrast to the dense white of that portion anterior to the anal segment. Anterior to the siphon there is a cuticular collar of a slightly darker shade.

The prolegs are formed by truncated projections, 6 in number, 3 on each side of the midventral line and extending laterally. Each protuberance is provided with a tuft of short, fine, brown hairs. These hairs appear to be surrounded by a secretory substance, which is slimy in character.

At the base of the siphon, beneath the cuticle on the dorsal side opposite the anal capsule, is a tiny structure which attracts attention on account of its movements and peculiar arrangement. In the newly hatched larva it is a process composed of 4 lustrous black disks arranged in two pairs, one in front of the other, and set in a mass of fat bodies. The larger of the disks, the anterior pair, is less than 0.1 millimeter in diameter. The movement of the process is similar to that of a pendulum, and is active only when the larva moves. With each molt these disks become smaller and increase in number. In the full-grown larva the process becomes a triangular mass of loosely arranged beaded disks. They appear to be mere specks of pigment beneath the skin, but their structure and action are so constant that either the process is characteristic of the species or investigators have overlooked or ignored them in other species.

## DESCRIPTION OF THE PUPARIUM

The average length is 18 millimeters, and width, 3.5 millimeters. The color is pale brown, the last 2 segments of the abdomen being slightly darker. The head tubercles are not clearly defined; color, dark brown. The prothoracic spiracular tubercle is slightly elevated, oblique; rima, salmon colored and crescentic in form.

The first abdominal spiracle is perfectly round and larger than the others, which are slightly ovoid; the rima of all the spiracles curves from above posteriorly.

The terminal abdominal segment shows a sexual distinction in the arrangement of the short spines midway on the ventral side, anterior to the terminal teeth. In the male 10 to 12 of these spines form a continuous serrated border. In the female the spines occur in two groups of 4 to 6 spines similar to those of the male, but separated by a space equal in width to that of one of the groups.

The terminal teeth of the posterior segment are arranged with 2 pairs close together on the dorsal side and 1 pair on the ventral side. These teeth are black-tipped and acute; all of them are directed slightly outward. The lateral teeth of the 2 dorsal pairs are the longest. The ventral pair is smaller and is set slightly in from the periphery of the segment.

After the final ecdysis which results in the formation of the puparium, the nymph, at first a light green, gradually changes to yellow. Upon the second day, the eye spots change from yellowish to pale brown, then to a chocolate color. Beginning with the third day the pads of the wings and the legs, at first light brown, assume the same color as the eyes. The chitinous pad enveloping the wing is densely opaque, so that only the plications of the developing wing can be discerned. Upon the penultimate day, the fifth or sixth usually, the abdomen, which heretofore has been a uniform yellow-brown, becomes striped with light orange and brown, which colors gradually deepen until the time of emergence.

### EMERGENCE FROM THE PUPARIUM

In emergence, the puparium which lies buried to some depth in the sand is invariably dragged to the surface where the final acts of emergence are completed. Two to three days prior to the act of emergence, the puparium shows considerable mobility when disturbed by handling or stimulated by light. Certain movements, which one learns through numerous observations to be characteristic, can be considered as actually premonitory. These occur usually from ten to twenty minutes prior to the breaking of the cuticle, and serve the observer as warning signs. If during this interval a low-power lens be focussed on the compound eye, the epidermis of the fly separating from its connective-tissue fastening of the puparium can easily be seen. This action resembles strikingly a wave of water moving between the walls of the puparium and the epidermis of the fly. It may be considered as the movements of a semiliquid layer between the fly and its puparium. Another movement, which can be observed within a few minutes after that previously described, is the momentary contraction and expansion of the sides of the abdomen between the two lateral ridges. This too, no doubt, is effective in tearing the connective-tissue lining to facilitate emergence. A few minutes later the anal end of the abdomen is torn loose from its fastening, and emergence of the fly begins.

Since the puparium is unrolled from the head, the compound eyes are soon exposed to view, so that the sex of the fly may be distinguished. The appendages, antennæ, palpi, and mouth parts are dimly visible. The head appendages are freed primarily by the spasmodic wriggling of the abdomen, but the labellum, which is seen to become turgid and flaccid in turn by the injection of air into, and withdrawal of air from, the "extensive tracheal sacs which lie in its cavities," and the erectile stomal disk through its pressure downward against the walls of the puparium, assist also in the process. That these head appendages assist effectually in the emergence is evident from the lines of cleavage in the enveloping membranes.

The puparium splits on the median line of the thorax; simultaneously the hood enveloping the head drops by a sternal hinge. The labellum can be seen still pressing upon the interior of the hood as the head emerges. Within a minute the wings are rent from their envelopes by the sturdy pressure of the legs, which have slid out of their sheaths simultaneously with the cleavage of the thorax. The legs directly assume their normal position, and the fly walks forth bodily, spreading its unplicated wings. The liberated wings show a clear expanse of unwrinkled membrane which at first is soft in texture and clear lead-colored throughout. Finally, the inflated abdomen appears in the dorsal slit, and at once is drawn clear of the encumbering puparium.

The time from the appearance of the head to the evacuation of the puparium requires less than two minutes. This time is increased a minute or two whenever the wing sticks to the lining of the puparium, resulting usually in a torn wing.

Directly after emergence the wings are shorter than the body, but, constantly vibrating, they gradually lengthen, whereupon they become hardened and prepared for flight. The fly does not spend any time preening itself as is the case with some of the Muscidæ at this stage. The time prior to flight is spent, however, in a clearing process. This begins with a copious discharge of meconium within three to five minutes after emerging. At first the defecation is performed at least five times per minute, then once per minute for a period of twelve minutes. At the end of this time the excretions become more watery in character. In the meantime the fly walks about in a restless manner, constantly vibrating the balancers and flapping its wings, while the distended abdomen becomes reduced to more normal proportions.

The meconium, which is deposited in large quantities, is in color pale brown, rapidly changing to amber, then becomes clear. The primary, heavier excretion appears decidedly oily in nature, when examined with the microscope.

In from fourteen to twenty minutes, voluntary flight takes place. This is at first tentative, the insect alighting upon the floor about a meter distant. After a minute of rest, flight is resumed, the fly escaping through the open window.

The puparium left behind shows certain points of cleavage which prove to be very constant. There is a dorsal slit on the median line of the thorax which extends nearly the length of the notum. Another slit extends midway across the orbital region through the genæ to the wing pouches. A third slit extends between the two wing envelopes, and a slight one behind the prothoracic spiracular tubercle.

In the 32 emergences recorded, the males preceded the females by an average of half a day. The males spent from three to seven days in the pupal stage, averaging five and one-half days, while this period required four to nine days with an average of six days in the female flies.

## GENERAL DESCRIPTION OF THE IMAGO

### MALE

The male is very distinct from the female, being smaller and having a larger head and different color markings.

The distinctly clavate palpi are shorter than in the female, only two-thirds as long as the labium; they are dirty white and fringed with moderately long black hairs.

The abdominal color markings take the form of a T of pale cadmium yellow in a field of burnt sienna, bordered with pale clay yellow. The area of the large facets of the eyes is colored

Roman sepia surrounded by an elliptical band of ultra ash gray. The field of small facets has a mauve fringe bounding an area of iridescent mauve and Prussian green.

Size: 14 to 15 millimeters.

Wing expanse: 25 to 28 millimeters.

### FEMALE

The front is narrow, converges slightly anteriorly; the color is golden, marked with a black callosity of irregular form.

The head is considerably smaller than that of the male; eyes iridescent mauve and Prussian green.

The palpi are prominently conical, as long as, or slightly longer than, the labium; the color is the same as in the male, mottled with short black hairs.

The abdomen is alternately striped with Cologne earth and pale clay yellow. The median stripe is pale clay yellow. In both sexes the thorax is indistinctly striped with pale clay yellow and pale brown, and the wings are transparent except the costal and subcostal cells which are pale brown.

Size: 15 to 17 millimeters.

Wing expanse: 26.5 to 29 millimeters.

### FEEDING HABITS OF THE IMAGO

The males in nature appear to derive the greater portion of their subsistence from gum exuded from trees, particularly rain trees; they can be found upon these trees at all hours, feeding whenever a gummy excrescence is present. In addition to the gum they lap the moisture found on the leaves in the early morning. In this connection Baldrey(3) found that *Tabanus* in India has a fondness for chestnut leaves, and sucks greedily any fluid thereon.

The females spend much of their time on trees in company with the males, feeding in a similar fashion. There is no doubt that this is the primary source of the female's food, but whether it is sufficient to stimulate egg laying is undetermined. Judging from the longevity of females kept under experimental conditions, it appears that a diet of blood is essential for the development of the eggs. For example, 200 flies kept individually in large flasks were fed daily on fresh gum of the rain tree. The records show that females outlived the males several days, and that the former lived a maximum of fourteen days. Females fed on monkeys and guinea pigs daily and permitted to feed as often as they desired were kept in similar flasks and under similar conditions as those in the first experiment. The average longevity of these proved to be ten days, and the maximum eighteen days. Captive flies were used in the first experiment and laboratory-bred flies in the second.

In considering the feeding habits of the female, it is deemed profitable to discuss the notes taken from observations made at close range with laboratory-bred flies fed on experimental animals. The behavior of flies bred in the laboratory indicates that the female does not feed on the day of its emergence. Thirty individuals tested in this regard refused to feed on the first day of emergence, 3 fed upon the second day, and the remainder took the initial feed upon the third and fourth days.

The insertion of the proboscis seems to be not the only means of obtaining blood, for, when abrasions of the skin of the animal exist, the fly may actually fill itself to the engorging point by means of its spongy labellum. In this process the latter organ is unassisted by the other mouth parts. The proboscis is a distinct appendage never coöperating with the lapping organ. The labellum is situated posterior to the piercing structures; it is held in this relation either when at rest or when active. When the labellum is in action, the stomal disks which constitute the lapping structure are unfolded downward and outward, bringing the pseudotrachea of the lapping area in contact with the moist surface of the skin. The female laps the blood offered in the same manner as it would any desirable liquid. This does not appear to be a usual method of obtaining blood, since it has never been observed under natural conditions. If it did occur in nature it is obvious that it might prove of importance in the mechanical transmission of animal diseases. This is at once apparent when one considers the area of the exposed surface of the lapping organ of this fly as compared with that of other flies infesting domestic animals.

Under experimental conditions *Tabanus striatus* bites usually not oftener than once in two days. A few instances have been noted of biting on consecutive days, but this is considered abnormal.

The process of inserting the various elements of the proboscis is difficult to ascertain since the large head conceals the biting parts when the vertex is lowered. In the process there are no movements which might be considered as premonitory. The fly when applied to the host quickly lowers its head, braces itself on its widely spread legs, and stabs its victim. The end of the abdomen is scarcely inclined, but settles in position parallel to the host's skin. The palpi, the position of which may be seen when the parts are withdrawn, are directed alongside the proboscis bow-like on either side of the puncture. These parts bend as the labium is thrust into the skin, and they probably aid by their elasticity in the withdrawal of the proboscis.

The head raises with a jerk in the withdrawal of the proboscis, and the punctured site is marked by a distinct blooddrop. Only a few seconds intervene prior to a repetition of the stab in an adjoining spot, and as many as 7 punctures may be made in a period of ten minutes. The biting may continue for as long as twenty-three minutes. The distention of the abdomen, only the anterior part of which is affected, gives no indication of the extent of satiation of the fly. They have been seen to feed with the abdomens bulging laterally with food, which bulging sometimes persists for several days, after which time the fly may bite once or even twice. The bites appear to be very painful to the host. The insect is never satisfied with a single bite, but makes several punctures before a complete meal is obtained. No doubt this is due in part to the interruptions caused by the tormented animal, and the fly may have acquired the habit of making short swift stabs in order to facilitate its escape from the host.

*Tabanus striatus,* although commonly termed a "horsefly," is found biting cattle and carabaos as well as horses. During over two years of personal observation, this fly has never been known to annoy man in the Philippine Islands. Reports of attacks from this fly can usually be attributed to the biting of *Stomoxys,* which is frequently annoying to the native caretakers of draft animals. Perhaps the carabao may be accepted as the host of choice of the Philippine horsefly. This is at once apparent when flies are very abundant. Many times I have seen sparsely haired carabaos with their bodies actually covered with droplets of dried blood resulting from the bites of tabanids. I have collected from a single carabao 61 specimens of *Tabanus striatus* in less than fifteen minutes.

## THE OCCURRENCE OF TABANUS STRIATUS

The time of day when this species of fly is most active is usually the same as that of other species of *Tabanus.* The fly begins its activities usually between 7 and 8 o'clock in the morning, and during the greatest heat of the day is found on the shady sides of houses and trees. Between the hours of 3.30 and 6 in the afternoon it is again active, resting for the night on the convenient rain trees. These trees have been observed to harbor

*Tabanus striatus* of both sexes at all hours of the day and far into the night. During the cooler part of the day the flies assemble on the trunk and main branches, but when it is sunny the upper limbs and leaves are resorted to; on windy days they may be found at all hours perched on the side of the trunk of the tree shielded from the wind. During their greatest prevalence they are found in large numbers indoors in the lightest portions of the room, usually perched on the windows.

The flies obtained for this biological study were collected from rain trees in the vicinity of the laboratory. These when gathered were identified and turned loose in the large breeding cage where two carabaos furnished the food required for further development. The daily supply of flies varied from 25 to 300 according to their prevalence and the skill of the Filipino collectors. Insect nets were used for trapping.

In 1912 the first appearance in this locality of horseflies in appreciable numbers was on August 15. From that time tabanids were collected daily at the rate of 25. August 15 to September 4 males predominated in the proportion of 2 or 3 to 1 female; and shortly afterward the female count increased to the proportion of 1 male to 1.7 female. There was a constant preponderance of females to the extent of 3 to 1 in October and 4 or 5 to 1 in December.

Beginning September 4, there was observed the first appearance of mammalian blood in flies collected from rain trees. The source of this blood, which showed itself in the dejecta and dissected stomachs of the females, was presumably a herd of 50 cattle which had been grazing on land along the lane of trees which harbored the flies. Although the cattle were present for eight days previously, blood was not taken until September 4. From September 4 to September 6 every lot of females collected was found to contain a preponderant number of individuals containing blood.

The first occurrence of oviposition was observed in a female collected in a lot of flies from this locality. It was removed from a tree September 10, and found laying eggs the following day, September 11. These eggs proved fertile, and were subjects of the life-history study described in this paper.

## SUMMARY

1. The eggs of *Tabanus striatus* Fabricus have been found in masses of from 270 to 425, laid mainly on particles of wood.

The incubation period has been determined to be from three to five days.

2. The behavior of the larva indicates that it is essentially an aquatic form. The insect in this stage has been found to be extremely cannibalistic. In some instances as high as 85 per cent of the brood has been destroyed by this means. They apparently prefer their kind to any other food; at least, there is no diminution of the practice even when an abundance of other food is present. There is shown to be a marked diversity in the development of larvæ of the same age. The larval period covers six weeks or longer.

3. In the ecdyses of *Tabanus striatus* there are 3 distinct molts; the final one, coming a considerable time after the larva is full-grown, results in the unveiling of the puparium. The puparium is formed beneath the molting skin of the full-grown larva. This stage lasts from three to seven days, with an average of five and one-half days, in the male; and from four to nine days, with an average of six days, in the female. In this period the male can be distinguished by the arrangement of the short spines anterior to the terminal teeth of the abdomen. These form a continuous serrated border of from 10 to 12 short spines. In the female these spines occur in two groups of from 4 to 6 each. Evidence of development of the adult fly is had in the changes of coloration visible through the puparium.

4. The imago emerges through an opening formed by a dorsal slit of the thorax and the fractured hood of the orbital region. There is a definite clearing process upon emerging. In from fourteen to twenty minutes after emergence the imago takes flight.

5. The process of feeding in the female fly is described. Both sexes have a lapping organ, in addition to which the female possesses a distinct piercing organ. In the latter sex two methods of procuring food are indicated.

6. All of the draft animals of the Philippines serve as hosts for *Tabanus striatus*. The carabao appears to be the host of choice.

7. The rain trees of this locality serve to harbor great numbers of resting horseflies. In considering methods of eradication, this fact should be considered.

8. The following table gives an outline of the minimum days required for the life cycle of flies of this species. From the time the eggs are laid to the emergence of a female fly a period of fifty-two days elapses.

TABLE II.—*Life-history chart of Tabanus striatus Fabricus.*

First observation of flies in nature, August 15.
First appearance of mammalian blood in the flies, September 4.

| Date. | Stages. | Period of development. |
|---|---|---|
|  |  | *Days.* |
| Sept. 11 | Beginning of egg laying | ---------- |
| Sept. 15 | Hatching of eggs | 4 |
| Sept. 26 | First molt | 15 |
| Oct. 9 | Second molt | 28 |
| Oct. 17 | Larva full-grown | 36 |
| Oct. 24 | Third molt | 43 |
| Oct. 24 | Puparium formed | 43 |
| Oct. 31 | Emergence of male fly | 50 |
| Nov. 2 | Emergence of female fly | 52 |
| Nov. 10 | Date of death of male | ---------- |
| Nov. 20 | Date of death of female | ---------- |

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(2) KING, H. H. Report on economic entomology. Third Report of the Wellcome Research Laboratories (1908), 213–214.

(3) BALDREY, F. S. H. The evolution of *Tr. evansi* through the fly: *Tabanus* and *Stomoxys* (1911). *Journ. Trop. Vet. Sci.* (1911), 6, 278.

# ILLUSTRATIONS

(Plate I; Plate IV, figs. 3 and 4; and Plate V, figs. 5 and 6, are from photographs by Boynton. Plates II, III, and VI; Plate IV, figs. 1 and 2; and Plate V, figs. 1 to 4, are from photographs by Cortes; Plate VII is from a colored drawing by Dimayuga.)

## PLATE I

FIG. 1. *Tabanus striatus* Fabricus depositing eggs on a flat surface. × 1.4.
2. A group of egg masses attached to bits of wood. × 1.9.
3. A mass of eggs photographed during the process of hatching. A few white bodies are seen emerging through the eggshells. The tracks made by escaping larvæ can be traced. × 5.
4. A mass of empty eggshells. × 6.6.

## PLATE II

FIG. 1. A group of *Tabanus* larvæ one hour after hatching. × 7.9.
2. An individual larva one day old. × 30.
3. The head capsule of a developed larva, showing the great hooks, stomal disk, and pharyngeal apophyses. × 15.3.
4. Full-grown larvæ. × 1.2.

## PLATE III

FIG. 1. The exuvium of the initial molt. × 8.5.
2. The molted skin of the second ecdysis. The shed head capsule is at one end and the cuticle of the anal siphon opposite. × 8.
3. The shed skin of the final process of molting. × 8.

## PLATE IV

FIG. 1. The last segment of the male puparium, showing the terminal teeth. × 12.4.
2. The same of the female. × 13.3.
3. The puparium just prior to the act of emergence. × 2.2.
4. The fly emerging from its puparium. × 2.7.

## PLATE V

FIG. 1. Head of the male fly. × 6.3.
2. Head of the female fly. × 6.3.
3. Mouth structures of the male. × 12.
4. Mouth structures of the female. × 10.5.
5. Male fly with wings spread, showing body markings. × 2.
6. Female fly with wings spread. × 2.

## PLATE VI

FIG. 1. A group of male and female *Tabanus* on a branch of the rain tree, feeding on the gummy excrescence. × 0.48.
2. Female flies infesting a native pony, showing their characteristic position and attitude of feeding.
3. Flies on the abdomen of a work bullock.

## PLATE VII

FIGS. 1–2. *Tabanus striatus* Fabricus in resting position. × 1.5.
3–4. Male and female flies with wings spread. × 2.4.

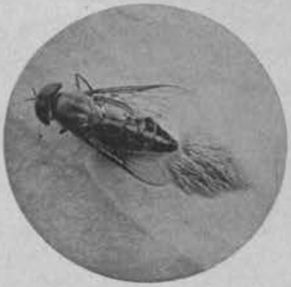


Fig. 1.

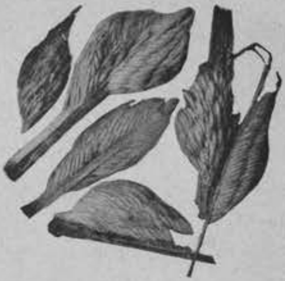
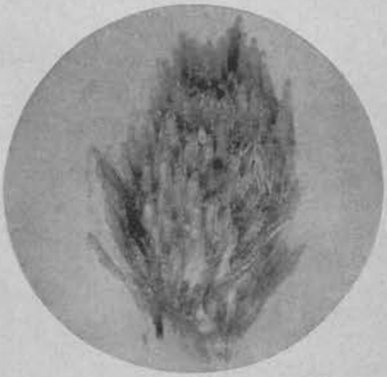


Fig. 2.



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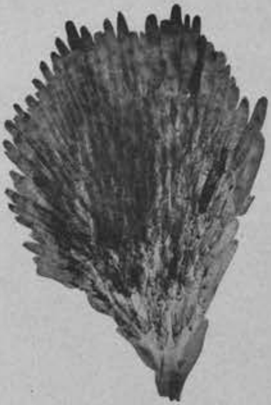
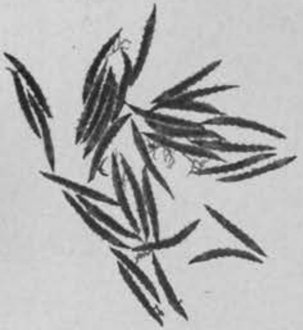


Fig. 4.

PLATE I.



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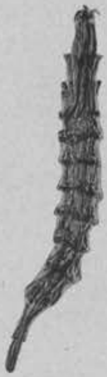

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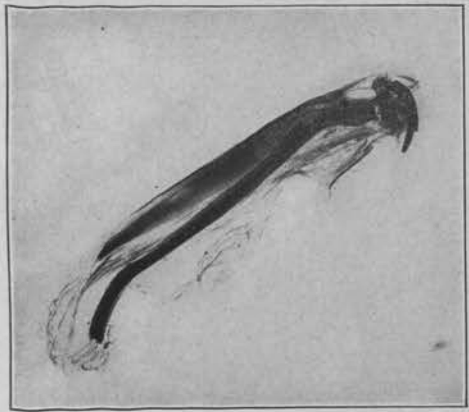

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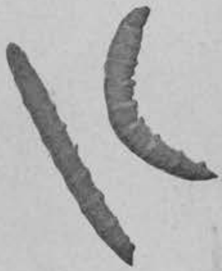
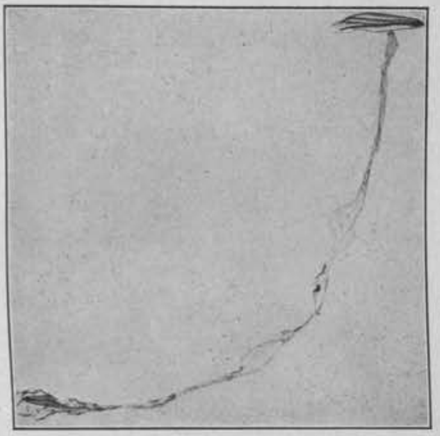


Fig. 4

PLATE II.



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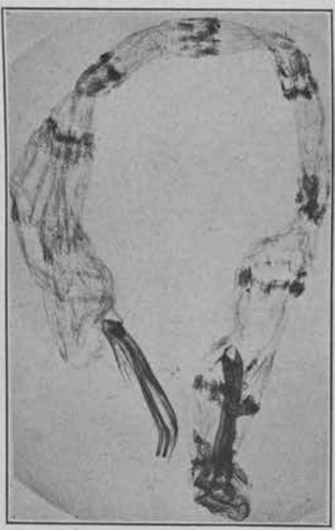

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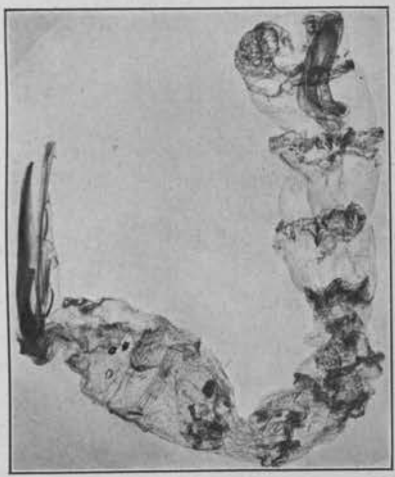
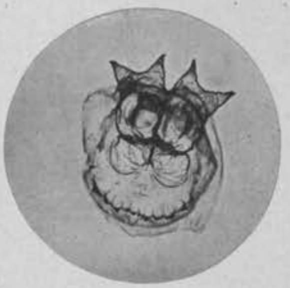


Fig. 3.

PLATE III.



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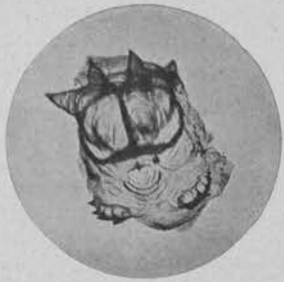

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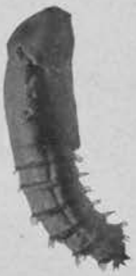

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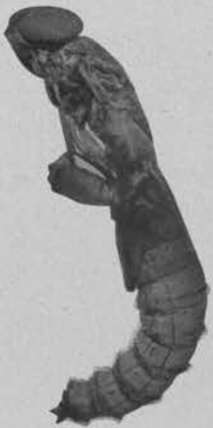
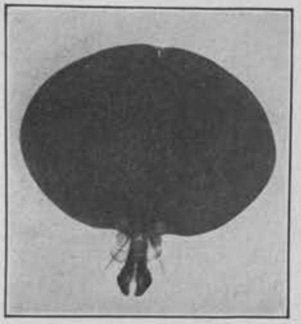


Fig. 4.

PLATE IV.



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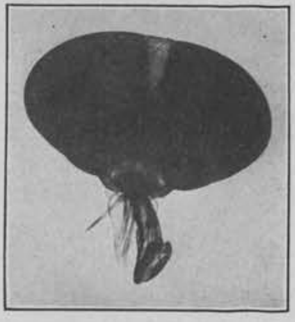
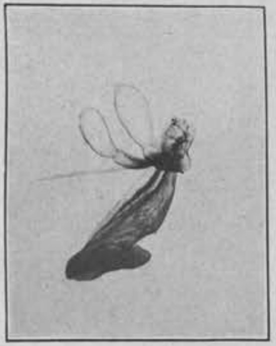


Fig. 2.



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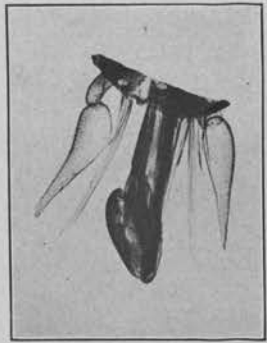

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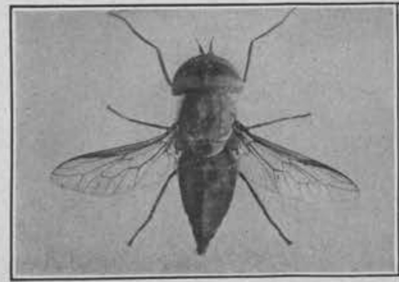

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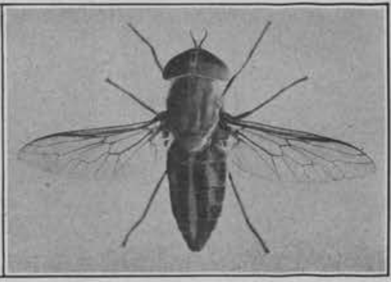

Fig. 6.

PLATE V.

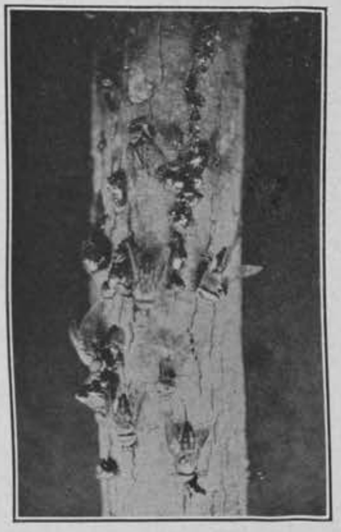

Fig. 1.

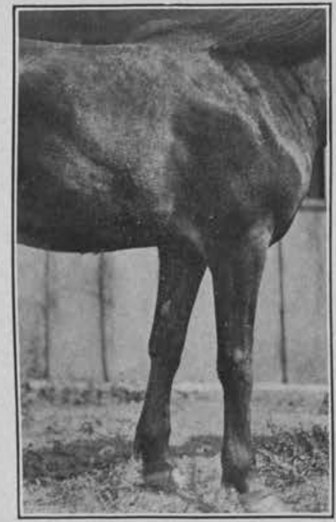

Fig. 2.

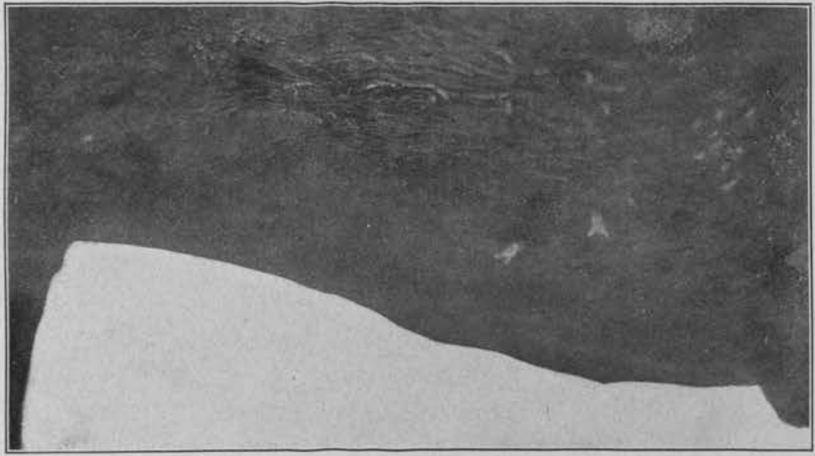

Fig. 3.

PLATE VI.

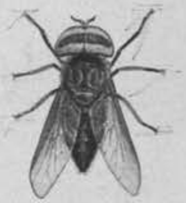

Fig. 1 ♂.

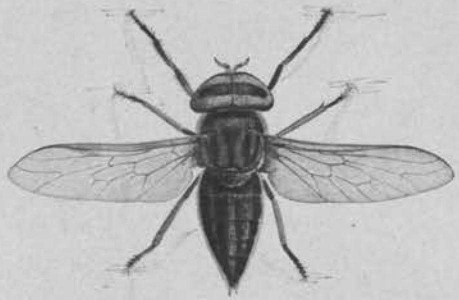

Fig. 3 ♂.

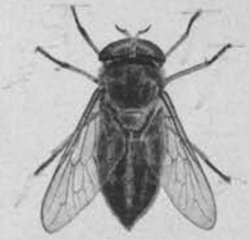

Fig. 2 ♀.

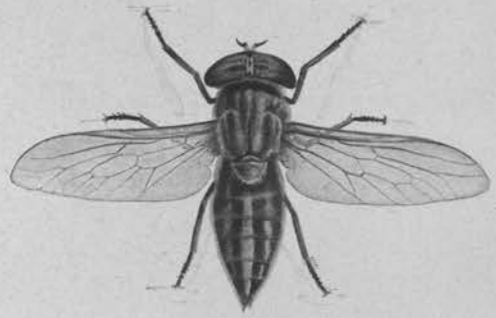

Fig. 4 ♀.

PLATE VII. TABANUS STRIATUS FABRICUS.

# THE MECHANICAL TRANSMISSION OF SURRA BY TABANUS STRIATUS FABRICUS[1]

By M. BRUIN MITZMAIN

(*From the Veterinary Division,*[2] *Bureau of Agriculture, Manila, P. I.*)

The Tabanidæ have hitherto not been investigated in connection with the transmission of surra in the Philippine Islands, as *Stomoxys calcitrans* has been the only carrier generally suspected. Recent work[3] has, however, practically eliminated the latter fly from further consideration as an important factor in surra dissemination. In no place has the transmission of any trypanosome infections through the bite of tabanid flies been proved with flies bred in the laboratory.

The geographical and seasonal distribution of *Tabanus striatus* have been recorded in the preceding article, and the status thereof marks this species as preëminently the most formidable bloodsucking fly in the Philippines.

The investigation of which this is a preliminary note has been conducted during the past year in the veterinary research laboratory at Alabang, Rizal Province, Luzon, with tabanid flies, which were for the most part bred from the egg; in some instances the flies were obtained from larvæ taken from their aquatic habitats; and in a few instances captured adult flies were employed.

In the present series *Tabanus striatus* was used in experiments on the direct transmission of surra. The flies were first allowed to bite an infected guinea pig or horse for not more than one minute, usually forty-five seconds; they were then transferred to a healthy animal and allowed to complete the meal without interruption. An interval of from five seconds to three minutes intervened during the transfer from the infected to the healthy animal.

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[1] To be published as Bulletin No. 28, Bureau of Agriculture of the Government of the Philippine Islands.

[2] Archibald R. Ward, chief.

[3] *This Journal, Sec. B* (1912), 7, 475.

In every instance the healthy animals used in the experiments were proved to be free from trypanosomes by rigorous quarantine and frequent microscopic examination of their blood. The mule and the horses employed in these experiments were kept for from six to eight months in the screened stable prior to their use for exposure to fly bites or blood inoculation to test the pathogenic nature of the trypanosome involved. The temperatures of these animals were registered morning and evening during the period of quarantine, preceding and following each experiment.

Monkeys, guinea pigs, and rabbits utilized in the experiments were quartered previously in fly-screened cages and declared surra free after ninety days, during which time blood examinations were made regularly once or twice weekly prior to the experiments.

Table I gives the data of these experiments.

TABLE I.—*Experiments on the mechanical transmission of surra by Tabanus striatus.*

| Date of experiment. | Trypanosomes in the infected host. | Flies employed. | Healthy animal used. | Result of experiment. |
|---|---|---|---|---|
| 1912 | | | | |
| Nov. 21 | Swarming | 1 | Guinea pig 87 | Negative. |
| 22 | Moderate | 1 | Guinea pig 92 | Negative. Subsequently reacted to surra by blood inoculation. |
| Dec. 12 | do | 1 | Guinea pig 111 | Negative. |
| 26 | do | 2 | Guinea pig 93 | Do. |
| Nov. 21 | Numerous | 1 | Monkey 5 | Do. |
| 22 | Swarming | 1 | Monkey P | Do. |
| 30 | Numerous | 1 | Monkey D | Do. |
| Dec. 1 | do | 1 | Monkey S | Do. |
| 28 | do.[a] | 3 | Monkey L | Positive on the eighth day. Dead Jan. 22. Blood produced surra in 2 guinea pigs and 1 horse. The latter died Mar. 1, 1913. |
| 1913 | | | | |
| Jan. 1 | Scanty | 2 | Monkey D | Negative. |
| 26 | Numerous | 2 | Horse 66 | Do. |
| 27 | do | 2 | Horse 69 | Do. |
| 28 | Swarming | 1 | Horse 277 | Do. |
| 30 | Numerous | 1 | Horse B-120 | Do. |
| Feb. 2 | Swarming[b] | 2 | Horse 50 | Positive on eighth day. One mule, 2 monkeys, and 2 guinea pigs reacted to inoculation of blood of horse 50. |
| 6 to 10 | Scanty to moderate.[b] | 6 | Horse 342 | Positive on ninth day. Disease reproduced by blood inoculation into 2 monkeys and 2 guinea pigs. |

[a] Infected guinea pig used.

[b] Infected horse used.

The three positive experiments are described in detail as follows:

The experiment in which monkey L became infected was conducted with flies bred from eggs. The source of this strain of surra was carabao 3182 which had been infected with surra for nearly one year previous to this experiment. Blood of this animal was inoculated into guinea pig 119 which showed infection in eight days, and was used for the present experiment, December 28, when its blood showed numerous trypanosomes. Three flies were applied individually in tubes to guinea pig 119 and allowed to feed from forty-five seconds to one minute and thirty seconds. They were then transferred to monkey L, after intervals of from twenty seconds to three minutes, and allowed to feed until satisfied. The flies fed on the latter animal five, sixteen, and twenty-one minutes, respectively.

From December 28 to January 8 no reaction was noted. The first high temperature, 40°.1, occurred on the evening of January 8, accompanied by a few trypanosomes in the peripheral circulation. The presence of trypanosomes continued daily, moderate to swarming in numbers, with several febrile periods until the animal's death on December 22, 1912.

Blood from the heart of monkey L was inoculated into horse 343 and guinea pigs 101 and 102. The latter showed infection upon the eighth and ninth days, respectively. Horse 343 had an abnormal temperature and a moderate number of trypanosomes in its blood upon the seventh day. The animal died March 1 with surra. At autopsy there was observed a general emaciated condition and enlargement of the spleen. The splenic pulp contained enormous quantities of trypanosomes as did the heart blood.

Horse 343 was also used as the blood donor in transmission experiments in which tabanids infected two other horses, namely, 50 and 342. The latter experiments were made in order to verify the previous one, in which a monkey contracted the disease. In only the first of the latter experiments were bred flies used. Two flies were permitted to bite horse 343 at a time when its blood was swarming with trypanosomes. The flies were interrupted in their biting in from forty to forty-five seconds and transferred to healthy horse 50. The infected flies bit after intervals of from five to fifteen seconds and were permitted to complete the feeding on horse 50. The latter was replaced in the fly-screened stable and examined daily. The initial rise of temperature was noted upon the eighth day, February 10, when a few surra organisms were found in the animal's blood. On the day following, horse 50 showed numerous trypanosomes in its blood and high temperature. Blood from this horse was inoculated into a mule, 2 monkeys, and 2 guinea pigs. The mule reacted with the usual symptoms after an incubation period of six days. Both monkeys had an incubation period of five days, and died of surra on the fourteenth and the fifteenth day, respectively. The 2 guinea pigs likewise became infected.

A second experiment was conducted with captured flies, surra horse 343 being used in this experiment. From February 6 to February 10, six flies in all were allowed to bite healthy horse 342 after they contaminated their proboscides with the blood of surra horse 343. The blood of the latter contained numerous trypanosomes upon only one occasion during the experiment; at other times the trypanosomes were scanty or moderate in numbers. The interval between the biting of the infected and the healthy horse was never more than twenty seconds, and the meal was completed in from four to eleven minutes on horse 342. An incubation period of nine days following the last bite elapsed before the healthy horse showed evidence of infection from the bites of the contaminated flies. On the evening of February 19 the temperature of horse 342 rose to 41°.1 C., and the blood showed a moderate number of trypanosomes. Upon the following day, when the trypanosomes were more numerous, blood from this animal was inoculated into 2 monkeys and 2 guinea pigs. The monkeys showed the first signs of infection on the seventh day and the guinea pigs on the eighth and ninth days. The 2 monkeys and the 2 guinea pigs were alive, but still infected on March 3, 1913.

### BITING EXPERIMENTS WITH ANIMALS IN A LARGE CAGE

An effort was made to induce flies to feed on a healthy and on infected animals kept together in a large screened cage. The results were negative, the flies dying in a few days when kept within the inclosure. The animals used were 2 surra-infected and 1 healthy carabao. The latter was separated from the others by a coarse-meshed wire partition. The flies were introduced daily into the common inclosure and were given ample opportunity to bite the animals exposed. From November 9 to December 22, 1911, 2,087 female tabanids were liberated in the cage. The animals were examined daily, and after the experiment the healthy animal was removed and observed. Fourteen months have elapsed and the exposed carabao remains normal. Two guinea pigs inoculated with its blood were alive and negative on April 12, 1913.

## HEREDITARY TRANSMISSION

As a precautionary measure it was thought advisable to eliminate the possibility, however remote, of the existence of hereditary transmission of trypanosomes in these flies.

In one experiment of this nature 74 flies were tested during two weeks after the emergence of the lot by allowing them to bite a healthy monkey. The eggs from which these flies developed had been laid August 14 by a fly which had fed twice on a monkey infected with surra and whose blood was swarming with trypanosomes. The following table contains the data resulting from allowing flies of this lot to feed on a healthy monkey. Monkey 5, which was examined daily during the experiment, showed no signs of infection and remained healthy until April 12, 1913.

TABLE II.—*Experiments to test the hereditary transmission of surra infection in Tabanus on monkey 5; results negative.*

| Date. | Flies tested. | Average time of feeding. |
|---|---|---|
| | | *Min.* |
| Nov. 5 | 1 | 4 |
| 7 | 3 | 7 |
| 8 | 3 | 7 |
| 9 | 4 | 9 |
| 10 | 3 | 6 |
| 12 | 5 | 6 |
| 13 | 10 | 9 |
| 14 | 9 | 10 |
| 15 | 6 | 8 |
| 16 | 8 | 6 |
| 17 | 4 | 7 |
| 18 | 7 | 6 |
| 19 | 6 | 4 |
| 20 | 5 | 4 |

## AN ATTEMPT TO TRANSMIT SURRA BY MEANS OTHER THAN BITING

It was observed that many flies of both sexes in feeding supplemented the sucking of the labium with the lapping of the spongy labellum. Usually these processes are independent, but not infrequently the female in attempting to bite the host will lap up any moisture present preparatory to inserting its proboscis. When an abraded surface is presented the majority of flies of this species are capable of nearly filling the stomach with blood without the aid of the proboscis. This has been observed in numerous instances in flies used in biting experiments, but has never been seen in flies attacking animals in the natural state.

Considering that there might be a remote possibility of conveyance of infection through this peculiarity in feeding habits, five experiments, based on this hypothesis, were attempted. The technique was the same in all of the 5 guinea pigs used. A highly infected guinea pig was used to contaminate the flies. A portion of the skin of infected and healthy animals was abraded with a razor and the flies applied individually in tubes. The fly was permitted to lap the blood from the abrasion on the infected animal for a minute or less and then transferred immediately to the healthy animal, where it was induced to apply its labellum for from five to ten minutes.

TABLE III.—*Attempts to transfer infection by the fly's labellum. Results negative.*[a]

| Date. | No. of guinea pig. | Flies applied. |
|---|---|---|
| Nov. 21 | 85 | 3 |
| Nov. 22 | 91 | 2 |
| Do | 110 | 2 |
| Nov. 23 | 104 | 4 |
| Nov. 24 | 123 | 3 |

[a] The experiment of guinea pig 110 was checked by subsequent inoculation to which the animal became infected.

The results of all the trials were negative, although it was ascertained that typical trypanosomes were present upon the labellum of one of the flies and in the stomach of another fly immediately after the experiment.

These experiments were supplemented by an experiment attempting to transfer infection to abrasions by the pulvilli of the contaminated fly. This also resulted negatively.

### LENGTH OF TIME TABANUS STRIATUS HARBORS TRYPANOSOMES

An attempt was made in this series of experiments to determine the maximum length of time surra organisms remain alive in the gut of the fly. The fly was fed in each instance on a guinea pig showing numerous trypanosomes in its blood. As noted in Table IV trypanosomes indistinguishable from surra organisms were found in suspensions from flies up to thirty hours after biting a sick animal. Beyond ten hours, inoculations of infected flies into susceptible animals were negative.

TABLE IV.—*Inoculations with suspensions of flies fed on infected animals.*

| Experiment No. | Animal inoculated. | Flies employed. | Interval after feeding of fly on infected animal. | Presence of trypanosomes in the fly. | Result. |
|---|---|---|---|---|---|
| | | | *Hours.* | | |
| 1 | Guinea pig 86 | 1 | 6 | Positive | Positive. |
| 2 | Guinea pig 89 | 1 | 10 | do | Do. |
| 3 | Guinea pig P. | 1 | [a] 24 | do | Negative. |
| 4 | Guinea pig 131 | 1 | 26 | do | Do. |
| 5 | Guinea pig 130 | 1 | 30 | do | Do. |
| 6 | Rabbit | 1 | 39 | do | Do. |
| 7 | Guinea pig 97 | 1 | 48 | Negative. | Do. |
| 8 | Guinea pig 98 | 1 | 48 | do | Do. |
| 9 | Guinea pig R. | 1 | 96 | do | Do. |

[a] The dejecta from this fly were found swarming with trypanosomes two and one-half hours after the fly bit the sick animal.

## CONCLUSIONS

1. *Tabanus striatus* Fabricus for the first time recorded has been found to play a rôle in the transmission of surra. Bred horseflies have been employed for the first time in such experiments. Errors resulting from naturally infected wild flies have thus been eliminated.

2. Three experiments were successful in the direct or mechanical transmission by "interrupted" feeding when only a short interval was allowed between the bites on infected and healthy animals. In 16 experiments the minimum number of flies with which the infection could be transmitted was 2.

3. Trypanosomes of surra were not found to be transmitted hereditarily in *Tabanus striatus* Fabricus.

4. The contaminated labellum of the fly does not appear to be a factor in the conveyance of infection.

5. The maximum length of time that *Trypanosoma evansi* has been demonstrated microscopically in the gut of this species of fly after feeding on infected blood is thirty hours; the organisms were found in the fly's dejecta two and one-half hours after biting the infected animal; and suspensions of flies, when injected subcutaneously, were found infective for animals for a period of ten hours after the flies had fed on infected blood.

# AXILLARY TERATOMA

By P. K. Gilman

*(From the Philippine General Hospital and the College of Medicine and Surgery, University of the Philippines)*

Two plates

While numerous examples of embryomata occurring in the more frequently involved portions of the body are constantly being reported, a review of the available literature fails to reveal a similar case to the one here described.

Teratomata are tumors frequently containing a number of different forms of tissue, bone, teeth, hair, skin, muscle, and glands. They are more frequently found at the lower end of the spine where the division occurs between the neural and gut portions of the neuroenteric tube, about the head, and in the generative organs. Tissue derived from any or all of the three layers of the blastoderm occurs in these tumors, and a preponderance of any one type of tissue in the growth has led not only to a classification of these growths, but, previous to the work of Wilms, to some confusion as well.

The work of Wilms(1) in 1895 and in 1899 on dermoid cysts of the ovary did a great deal to clear up many questions concerning the anatomical peculiarities of these tumors and render their grouping possible. He showed these tumors to be complicated structures containing derivatives from all of the three blastodermic layers, applied the term embryomata, and believed them peculiar to the ovary, developing from the growth of an unimpregnated ovum.

It was shown later that embryomata of the ovary differ in no essential manner from embryomata occurring elsewhere in the body, thus doing away with the earlier idea of Wilms of ovum origin.

A few references from a large literature are cited to illustrate the more frequent situations in which these growths occur.

Weigert(2) studied a 3.5-centimeter teratoma of the pituitary body occurring in a boy of 14 years. The growth contained epidermis, hair follicles, hair, sebaceous glands, fat, cartilage, and smooth muscle.

Ford(3) reports the removal of a large teratoma from the right side of the face and neck of a mulatto woman aged 40 years. The tumor had been observed since the age of 18 years. It had increased rapidly in size for two years prior to its removal.

Musick(4) describes in detail a case of teratoma of the right lobe of the liver, causing death in an infant of 2 months. In this case structures were found having their origin in the mesoderm and entoderm, but not in the ectoderm.

Mandelbaum(5) reports a case of dermoid cyst of the mediastinum in a Russian woman of 30 years, and has collected 36 other cases from the literature. He suggests as a result of study of the cases reported and the histological findings in his own case that these growths be classified with the teratomata and further suggests the following groupings:

I. True dermoids containing only ectodermal structures.
II. Teratomata, dermoids with the addition of structures from the entoderm and mesoderm.
III. True dermoids or teratomata, with the addition of tumor formations.

Anspach(6) summarizes the opinion of Wilms and others regarding teratomata of the ovary, and reports a case of ovarian teratoma with a large proportion of thyroid gland tissue in the dermoid prominence removed from a woman of 36 years.

Ewing,(7) from a study of 2 cases of testicular tumors, concludes that teratomata of this region developing unequally or in a one-sided manner give rise to the myxomata, myomata, chondromata, carcinomata, and large round-celled sarcomata or carcinomata of the testes.

Bergmann(8) reports 5 cases of congenital sacral teratomata operated upon in children. One of the cases possessed malignant characteristics with metastasis.

## CASE REPORT

Mateo Serrer, Filipino, age 7 years. Operation, August 9, 1912.

Family history negative. Eleven brothers and sisters died, some of smallpox and some of beriberi.

Patient was born without medical attendance; delivery was normal. Child was breast fed at irregular hours, but with good success. Dentition was normal. There were no previous diseases with the exception of mild fevers.

Present illness is congenital, a swelling having been noted under the right arm shortly after birth. This swelling has increased slowly but steadily in each diameter until a few days ago when the patient had a slight fever and the tumor became red and increased noticeably in size.

The present condition of the child is, in general, good. He is fairly well nourished, although somewhat underdeveloped. The general examination is without especial interest.

Occupying the right axilla and limiting considerably the movements of the arm in all directions is a tumor measuring about 12 centimeters in diameter, with a general spherical form. The growth as a whole is soft and elastic and is movable, although evidently attached to the apex of the axilla and upper portions of the chest wall; is not tender nor painful. The skin over the mass is more or less adherent in several linear areas, one of these on the external surface being reddened and inflamed. The vessels of the skin over the growth show great distention and considerable venous stasis. The tumor evidently contains fluid under considerable tension, and over the posterior portion of the wall of the cyst gives a sensation as though considerable fibrous thickening had taken place with probably a deposition of some mineral salts.

Under chloroform anæsthesia, an oblique incision was made over the tumor and the skin and subcutaneous tissues were stripped back from the cyst wall proper. The growth shelled out readily over nearly the entire surface, difficulty being experienced only in the apex of the axilla where the artery, several branches of the vein, and most of the nerve branches were enveloped in the fibrous capsule of the cyst and over the fifth rib, the periosteum of which was intimately connected with the wall.

The wound was closed with a cigarette drain and the child, although having taken the anæsthetic badly, made an uninterrupted recovery.

The tumor was sent to the pathological laboratory, and was examined by Dr. B. C. Crowell, whose report follows:

Specimen consists of a mass of tissue removed from the right axilla. This is irregularly globular in shape, and measures about 12 centimeters in diameter. There are some fibrous tags on the outer surface, and it is apparently cystic, being formed of larger and smaller cysts, some as large as 4 or 5 centimeters in diameter. A small portion of the fluid of the cyst was aspirated immediately after the specimen was received, and was seen to be clear and yellowish in color. The specimen was fixed intact, and after fixation was divided into two equal portions. The cysts of which it is made up are filled with a partially coagulated greenish fluid (after fixation). On removal of this fluid, one large cyst measuring 6 centimeters in diameter and other smaller cysts are seen; the walls of these are smooth and pale, and the larger cysts have thin walls. Across one of the larger cysts stretches a band of soft, gelatinous tissue about 2 centimeters in diameter. At the periphery of one-half of the cut section are seen numerous small cysts in the wall of the mass, these being separated by a pale tissue. Some of these cysts have a clear content, and in some it is yellowish and rather firm, and again in others of a deep red color.

Microscopically, sections taken from the walls of the cysts are seen to contain numerous fibrous connective tissue, muscular and adipose elements irregularly arranged, and in their midst are numerous, very thick-walled blood vessels. One small portion of a lymph node is also seen in one section imbedded in the wall. There are also numerous small cystic spaces, some of which are filled with blood and others filled with a pink, gelatinous material. The walls of these smaller cysts are formed by very low, flat, epithelial cells, the nuclei of which are rounded in many places. There is also considerable leucocytic infiltration of different parts of the mass. The walls of the larger cysts are of the same character as the walls of the small cysts.

Teratoma of mesodermal origin or autochthonous monophyllic teratoblastoma of mesodermal origin (Adami).

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# ILLUSTRATIONS

## PLATE I

FIG. 1. Anterior view of axillary teratoma.
2. Posterior view of same.

## PLATE II

FIG. 1. Tumor after removal.
2. Same tumor after section.

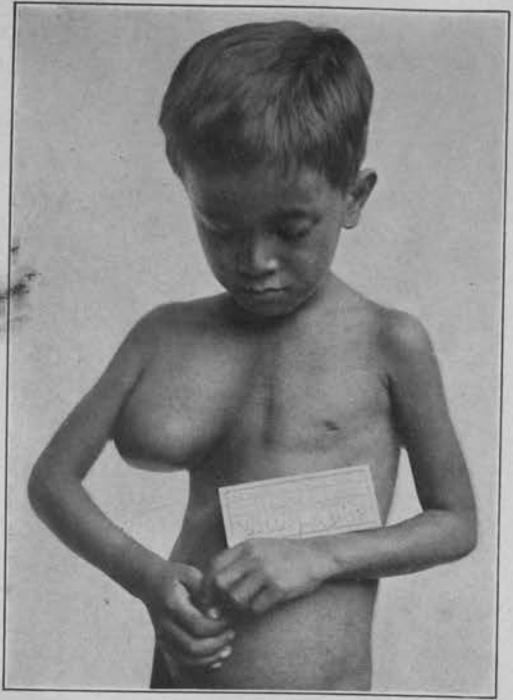

Fig. 1. Anterior view of axillary teratoma.

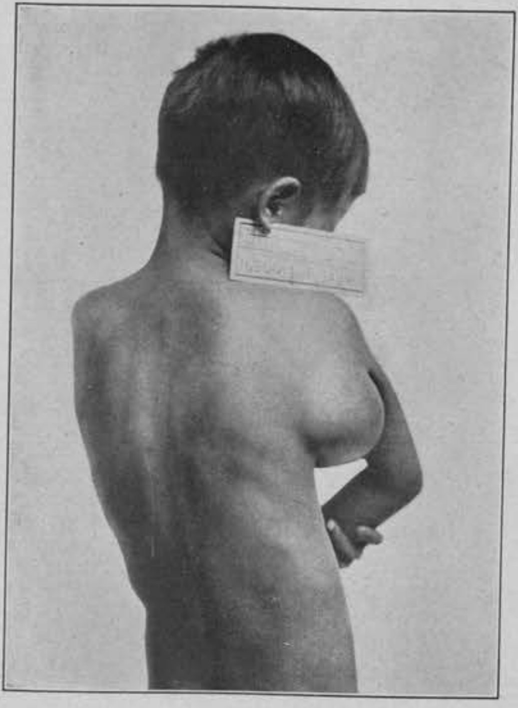

Fig. 2. Posterior view of same.

PLATE I.

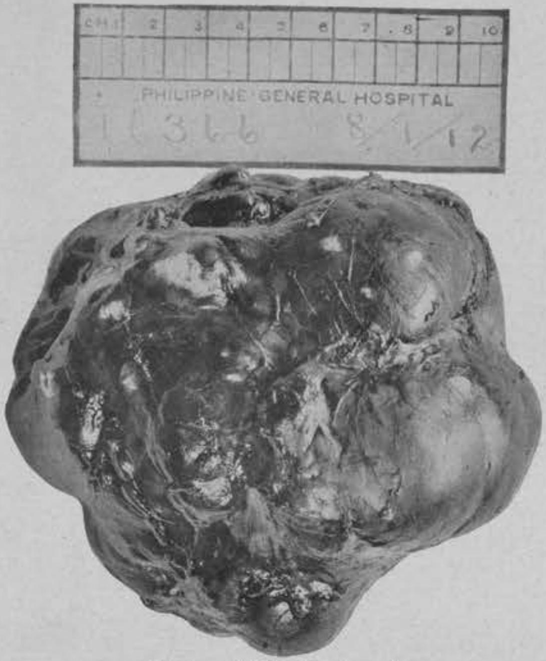

Fig. 1. Tumor after removal.

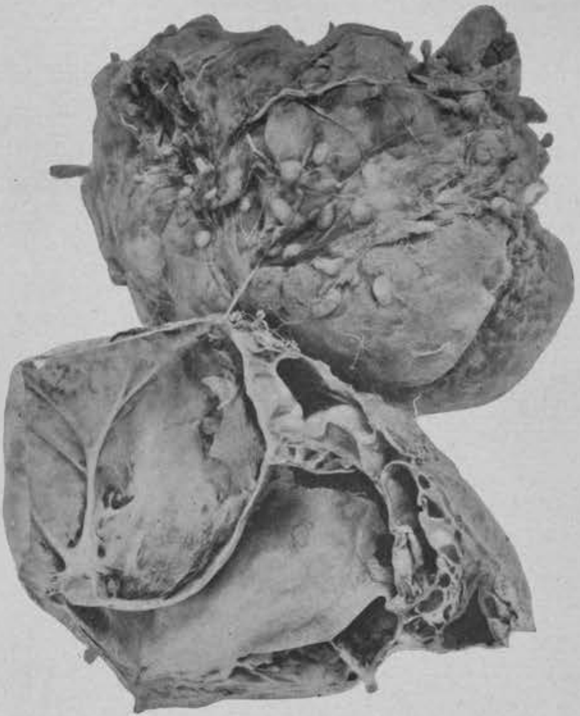

Fig. 2. Same tumor after section.

PLATE II.

# A NOTE UPON STRANGLES IN THE PHILIPPINE ISLANDS

By William Hutchins Boynton

(*From the Veterinary Division,*[1] *Bureau of Agriculture, Manila, P. I.*)

It is believed by some members of the medical profession that streptococcic infection in man is of rare occurrence in the Philippine Islands, and, when present, is mild in effect.

Musgrave [2] states that—

> The most logical explanation of the ease with which infection may be avoided in both surgery and obstetrics is the rarity of virulent streptococci, such organisms seldom being encountered in laboratory work, in the morgues or in clinical work in the hospitals.
>
> Streptococcic septicemia, streptococcic cellulitis, erysipelas, scarlet fever, and even streptococcic sore throat are extremely rare conditions in the Philippine Islands, and streptococcic metritis and peritonitis following child birth have not been encountered in autopsy work in Manila.

Since the diseases prevailing among the domesticated animals have not been worked out to any great extent here, it was not positively known whether or not they suffer from such infection.

The existence of strangles or distemper in horses appears to have attracted very little attention in the Philippines, and, although it has been recognized several times by veterinarians, the diagnosis in such instances was based entirely upon clinical symptoms, the causative agent not having been isolated and identified.

In searching for official reports on strangles in the Bureau of Agriculture files, none could be found, but by personal inquiry the writer has been able to gain some data as to its prevalence.

During the months of February, March, and April, 1909, shortly after the arrival of some old Army mares for breeding purposes at the Alabang stock farm, there was an outbreak of strangles which killed several colts, and ran a severe course in many others. By the strenuous work of the men in charge, the infection was cleaned up and has not appeared since.

---

[1] Archibald R. Ward, chief.

[2] Musgrave, W. E., Aseptic midwifery in Manila, *Bull. Manila Med. Soc.* (1910), 2, 134.

There have been several outbreaks of this disease at the Trinidad stock farm during which several colts and older horses have died. Veterinarians who have worked in Manila say strangles is prevalent among the carromata horses, and also occurs frequently in the city stables. A few years ago this disease is said to have killed one of the finest race horses in the Philippine Islands.

Strangles appeared among the horses at the Singalong experiment station in Manila about March 10, 1911, and on March 26, horses, which had apparently not been affected by the disease during this outbreak, were shipped from Singalong to Trinidad. About April 15 the disease was observed among the animals at the Trinidad stock farm, but, as the disease first appeared in a pony belonging to an employee at the experiment station, it was thought probable that the infection was brought in by this pony rather than by the horses from Manila.

On April 3, 1911, the animal husbandry division of the Bureau of Agriculture shipped 11 horses suffering with osteoporosis from the Trinidad stock farm to the veterinary research laboratory at Alabang. The animals were transported in stock cars, arriving at Alabang in apparently good condition, except for the deformities caused by osteoporosis. They were placed in a shed previously occupied by cattle immunized to rinderpest, which were used in the production of antirinderpest serum. Each horse was tied in a separate stall, but could easily reach over into its neighbor's manger on either side.

*Case 1.*—April 10, 1911, the writer's attention was called to a horse, 1 year old, which had a slight purulent discharge from its left nostril, the left submaxillary gland being slightly enlarged. The animal was immediately separated from the rest, and its manger thoroughly disinfected. On April 16 this animal died. Post-mortem examination revealed typical osteoporosis lesions, the right femur being completely fractured about 7 centimeters from its head, and practically all the bones of the body were more or less affected, which was undoubtedly the cause of death. At the time of death, the discharge from the nostril had practically ceased, but the affected gland was somewhat enlarged. Neither culture or smear preparations were made from the discharge or gland of this animal.

*Case 2.*—April 17, a horse, 1 year old, which stood in the stall adjoining that of horse 1 had a purulent discharge from both nostrils. The animal was immediately isolated, and the stall and manger thoroughly disinfected. On the 19th, the submaxillary glands were enormously enlarged. Four days later an abscess broke, discharging a thick yellowish pus, and on the 26th two more abscesses broke. The horse was placed on the operating table, and two more abscesses were opened with aseptic precautions. From these, cultures were made on agar-agar and in plain bouillon. Several smear preparations were made and, after staining, showed upon microscopic examination large numbers of cocci and a few short chains containing from 3 to 4 elements each. On the 28th, the agar culture showed several colonies of *Micrococcus pyogenes aureus* and *albus*. Besides these there were a few small grayish colonies which, upon microscopic examination of hanging-drop and stained smear preparations, proved to be streptococci. Long chains of streptococci were also found present in the bouillon culture but were mixed with numerous micrococci. Subcultures on agar and in bouillon were made from the small agar colonies. On April 29 the subcultures in both media revealed pure cultures of streptococci.

On May 1, 1 cubic centimeter of bouillon culture was injected subcutaneously into each of 2 rabbits and 2 guinea pigs, and 5 cubic centimeters subcutaneously into the neck of a horse 1 year of age. The rabbits and guinea pigs suffered no ill effects from the injection. The horse on the second day after injection showed a large swollen area around the point of inoculation which was very tender. On the fifth day the swelling was 14 centimeters in diameter. The lymphatic vessels extending from it were swollen, giving an identical picture of a typical, local mallein reaction in a glandered horse. On the eighth day the abscess thus formed broke and discharged a large amount of yellowish blood-stained pus, which upon microscopic examination of stained smear preparation revealed large numbers of streptococci in short chains.

At the time the abscess broke the horse ate very little and had a depressed appearance, lying down the greater part of the time. In a couple of days it regained its appetite, the swelling gradually subsided, and complete healing took place in fifteen days. The neighboring lymphatic glands were observed frequently, but no sign of enlargement or tenderness could be detected.

From all appearances, this inoculated horse had a mild attack of strangles, the infection remaining localized at the point of inoculation.

*Case 3.*—On April 25, a 2-year-old horse, which occupied the stall next to that of horse 2, was observed to have a slight discharge from both nostrils. It was immediately isolated, and the premises thoroughly disinfected. In three days it had a marked discharge from both nostrils, and both submaxillary glands were considerably enlarged. After ten days' duration the discharge ceased, and the glands gradually returned to normal size. A few short chains of streptococci were found on microscopic examination of the nasal discharge. In fourteen days this animal had apparently recovered from the attack.

*Case 4.*—On May 3, a small black mare, 1 year old, whose stall was next to that of horse 3, was observed to have a discharge from the right nostril. The animal was immediately isolated, and the premises thoroughly disinfected. The following day there was observed a discharge from both nostrils, and the submaxillary glands were slightly enlarged. At the end of ten days the discharge from both nostrils had ceased, and the glands were practically normal in size. Recovery was apparently complete in fourteen days.

## CONCLUSIONS

1. From the results derived from the cultures and from microscopic examinations of the purulent discharges, it is evident that streptococcic infection exists in horses in the Philippine Islands.

2. Since bouillon cultures had no effect on rabbits and guinea pigs when inoculated subcutaneously, and did have decided effect upon a horse, it proves conclusively that the organism isolated was *Streptococcus equi*. No white mice were on hand, so the virulence of the culture could not be tested on them.

3. From the information gained through inquiry, it is very evident that strangles is a widespread disease among horses in the Islands, an interesting fact in view of the reputed rarity of streptococcic infections in man.

# A FURTHER NOTE UPON THE INFLUENCE OF ATMOSPHERIC TEMPERATURE UPON THE SPREAD OF PNEUMONIC PLAGUE

By OSCAR TEAGUE[1]

(*From Cornell University Medical College, New York*)

Plague has raged in India for almost two decades, and has carried off several millions of victims. The epidemic has remained essentially bubonic in type. Although 1 per cent or more of all the cases have been plague pneumonias, these numerous scattered cases have not led to epidemics of pneumonic plague. That plague pneumonia may assume epidemic proportions under certain circumstances was demonstrated most conclusively in Manchuria during the winter of 1910–1911 by one of the most virulent epidemics of modern times. Teague and Barber[2] recently offered the following explanation of the rapid spread of pneumonic plague in Manchuria and the failure to spread in India.

> We believe we are justified in concluding from these experiments that were the plague organisms sprayed under similar conditions they would persist longer than cholera vibrios, but a shorter time than prodigiosus bacilli. Hence, it seems probable that the plague bacilli contained in fine droplets of pneumonic-plague sputum would suffer death from drying in a few minutes unless they were suspended in an atmosphere with an extremely small water deficit. Infection in pneumonic plague follows the inhalation of droplets of pneumonic sputum and obviously the longer these droplets remain suspended in the air, the greater is the danger of infection. As has just been stated, these fine droplets disappear very quickly except when they are suspended in an atmosphere with a very small water deficit. Such an atmosphere is, under ordinary circumstances, of common occurrence in very cold climates, whereas it is extremely rare in warm ones. Hence, since the droplets of sputum persist longer, the plague bacilli remain alive longer in the air, and there is a greater tendency for the disease to spread in cold climates than in warm ones.

During the Manchurian epidemic the temperature at Harbin (where the great majority of deaths occurred) ranged between

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[1] Assistant in experimental pathology, Cornell University Medical College, New York.

[2] *This Journal, Sec. B* (1912), 7, 172.

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> We believe we are justified in concluding from these experiments that were the plague organisms sprayed under similar conditions they would persist longer than cholera vibrios, but a shorter time than prodigiosus bacilli. Hence, it seems probable that the plague bacilli contained in fine droplets of pneumonic-plague sputum would suffer death from drying in a few minutes unless they were suspended in an atmosphere with an extremely small water deficit. Infection in pneumonic plague follows the inhalation of droplets of pneumonic sputum and obviously the longer these droplets remain suspended in the air, the greater is the danger of infection. As has just been stated, these fine droplets disappear very quickly except when they are suspended in an atmosphere with a very small water deficit. Such an atmosphere is, under ordinary circumstances, of common occurrence in very cold climates, whereas it is extremely rare in warm ones. Hence, since the droplets of sputum persist longer, the plague bacilli remain alive longer in the air, and there is a greater tendency for the disease to spread in cold climates than in warm ones.

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—9° C. and —32° C. However, in considering the above explanation one must bear in mind that in most instances infection almost surely takes place indoors, whereas these low temperatures were measured in the open. Furthermore, a well-heated room with a low outside temperature affords a greater water deficit than a similar room with a high outside temperature. Hence, if the lodging houses in Harbin were well heated during the plague epidemic, there would remain but little basis for the above explanation.

As no records of indoor temperatures during the epidemic are available, I have endeavored to secure temperatures of inns and lodging houses in Harbin in which plague patients were found at the same season of the year that the epidemic occurred. For this purpose I forwarded blanks to the American minister at Peking with the request that he have them filled out by persons known to be reliable. As a result, the following observations were made at the request of the American consul at Harbin by Dr. T. N. Tang, thanks to the kindness of Dr. Wu Lien Teh,[3] director of the North Manchurian Plague Prevention Service.

From Table I it is seen that 9 (Nos. 23, 24, 28, 29, 35, 36, 37, 38, and 39) of the houses investigated showed temperatures of 6° C. or less in at least one of the observations and 5 of these houses were known to have harbored plague cases during the epidemic. In 11 additional houses (Nos. 1, 2, 4, 9, 12, 17, 26, 31, 32, 33, and 41) temperatures of 10° C. or lower were recorded.

Therefore, it is clear that in Harbin there are native inns and lodging houses which during February are very inadequately heated and consequently must contain an atmosphere with a very low water deficit.

Since the publication of the article by Teague and Barber,

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[3] Dr. Wu Lien Teh has also furnished the following information, which has a definite bearing upon the subject under discussion:

"The native dwellings in Harbin are heated by brick stoves after the Russian style (very few); iron stoves in which coal is usually burnt; *kangs* (*i. e.*, large rectangular brick structures 2½ feet above the ground, in which the people sit and rest) heated usually by millet stalks; and open charcoal pans without chimneys.

"The windows usually consist of a wooden framework pasted with thin paper, thus letting in very little light. Beyond the doors and the cracks in the windows, walls, and roofs, there is seldom any ventilation inside the dwellings."

Rau[4] has described a small epidemic of pneumonic plague at Santa Maria in the southern part of Brazil. For four years isolated cases of bubonic plague had occurred in Santa Maria at intervals of weeks or months. On July 21, 1912, there suddenly appeared a case of pneumonic plague which was followed in the course of the next three weeks by 17 other cases, all ending fatally.

It is worthy of note that these pneumonic cases occurred during the cool season and that the houses in Santa Maria are not heated artificially. (Personal communication from Doctor Rau.)

Next to Harbin, Changchun suffered most during the epidemic. After Doctor Tang had made the observations here recorded for Harbin, he was sent by Doctor Wu to Changchun, where he noted the temperatures recorded in Table II.

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[4] *Deutsche med. Wochenschr.* (1912), 38, 2314.

TABLE I.—*Temperature observations*

| No. of observation. | Variety of dwelling. | Description of building. | Approximate size of room. | | |
|---|---|---|---|---|---|
| | | | Length. | Breadth. | Height. |
| | | | *Feet.* | *Feet.* | *Feet.* |
| 1 a<br>b | Native inn | Mud-walled room; old, no windows; native kangs on both sides; ground of room about 1 foot lower than that outside the door; earth floor. | 23 | 19 | 12 |
| 2 a<br>b | do | Brick wall outside; mud inside; earth floor; 1 window; kangs on both sides leaving passage in the middle. | 19 | 16 | 9 |
| 3 a<br>b | do | Earth floor; 6 paper windows; kangs on both sides. | 26 | 19 | 7 |
| 4 a<br>b | do | Plank floor; native kangs on both sides; 2 paper windows. | 23 | 20 | 8 |
| 5 a<br>b | do | Earth floor | 18.5 | 16.5 | 9.5 |
| 6 a<br>b | do | Plank floor; kangs on both sides of room; glass windows. | 17 | 17 | 7 |
| 7 a<br>b | do | Plank floor | 16 | 19 | 7 |
| 8 a<br>b | do | Earth floor; kangs | 19 | 19 | 7 |
| 9-a | do | Earth floor; mud and straw wall; 4 paper windows. | 19 | 19 | 10 |
| 10 a<br>b | do | Plank floor; 1 window | 9 | 19 | 9 |
| 11 a<br>b | do | Plank floor; kangs on both sides | 23 | 21 | 9 |
| 12 a<br>b | do | Earth floor; 4 windows | 19 | 19 | 9 |
| 13 a<br>b | do | Earth floor; 2 windows | 21 | 12 | 9 |
| 14 a<br>b | do | Plank floor; 1 window | 7 | 12 | 12 |
| 15 a<br>b | do | Brick floor; 1 paper window | 21 | 21 | 8 |
| 16 a<br>b | Private house | Earth floor; kangs; 2 paper windows | 19 | 7 | 8 |
| 17 a<br>b | Native inn | Earth floor; 4 windows; mud wall | 23 | 19 | 7 |
| 18 a<br>b | Shop | Earth floor; 2 windows on either side; kangs on both sides. | 10 | 21 | 7 |
| 19 a<br>b | Native inn | Plank floor; kangs on both sides; 3 paper windows. | 28 | 19 | 9 |
| 20 a<br>b | do | Earth floor; kangs on both sides; 8 windows. | 28 | 19 | 9 |
| 21 a<br>b | do | Earth floor; kangs on both sides; 2 paper windows. | 19 | 16 | 7 |
| 22 a<br>b | do | Plank floor; paper windows | 40 | 19 | 9 |

*in Fuchiatien (Harbin).*[a]

| Occupants at the time. | How heated. | Occurrence or nonoccurrence of plague. | Temperature. | | Date. | Time of day taken. | |
|---|---|---|---|---|---|---|---|
| | | | Inside of room. | Outside of room. | | | |
| | | | °C. | °C. | 1913. | a. m. | p. m. |
| 11 | Brick stove with chimney. | Not sure | 10 | − 3 | Feb. 2 | 12.00 | ----- |
| 15 | | | 15 | − 4 | | ----- | 9.30 |
| 11 | do | do | 7 | − 2 | do | ----- | 1.00 |
| 12 | | | 10 | − 4 | | ----- | 10.00 |
| 12 | do | (?) | 12 | − 2 | do | ----- | 2.30 |
| 12 | | | 11 | − 5 | | ----- | 11.00 |
| 14 | Open charcoal pan | (?) | 10 | − 2 | do | 12.00 | ----- |
| 14 | | | 14 | − 5 | | ----- | 4.00 |
| 12 | Stove with chimney | (?) | 17 | − 2 | do | ----- | 2.00 |
| 12 | | | 22 | − 5 | | ----- | 10.00 |
| 22 | Open charcoal pan | (?) | 16 | − 3 | Feb. 3 | ----- | 2.00 |
| 8 | | | 22 | −10 | | ----- | 10.00 |
| 8 | Kangs | (?) | 16 | − 3 | do | ----- | 3.00 |
| 9 | | | 17 | −10 | | ----- | 11.00 |
| 8 | do | (?) | 14 | − 4 | do | ----- | 3.00 |
| ----- | | | ----- | ----- | | ----- | ----- |
| 14 | Chimney stove | (?) | 10 | − 3 | do | ----- | 1.00 |
| 14 | | | 11 | − 7 | | ----- | 8.00 |
| 7 | do | (?) | 12 | − 3 | do | ----- | 3.00 |
| 7 | | | 15 | − 7 | | ----- | 9.00 |
| 6 | Kangs | (?) | 16 | − 4 | do | ----- | 4.00 |
| ----- | | | 16 | − 8 | | ----- | 10.00 |
| 14 | Stove with chimney | (?) | 10 | − 3 | Feb. 4 | ----- | 3.00 |
| ----- | | | 13 | − 7 | | ----- | 9.00 |
| 8 | do | (?) | 13 | − 8 | Feb. 5 | ----- | 4.00 |
| 8 | | | 18 | −15 | | ----- | 8.00 |
| 5 | Open charcoal pan | (?) | 15 | − 9 | do | ----- | 5.00 |
| 5 | | | 18 | −17 | | ----- | 9.00 |
| ----- | Stove with chimney | (?) | ----- | ----- | do | ----- | ----- |
| 9 | | | 21 | − 8 | | ----- | 8.00 |
| 2 | do | (?) | 16 | −15 | Feb. 6 | ----- | 2.00 |
| 2 | | | 17 | −19 | | ----- | 9.00 |
| 12 | do | (?) | 9 | −19 | Feb. 7 | ----- | 1.00 |
| 11 | | | 8 | −24 | | ----- | 9.00 |
| 8 | do | (?) | 11 | −16 | do | ----- | 2.00 |
| 2 | | | 15 | −24 | | ----- | 8.00 |
| 11 | do | (?) | 13 | −17 | do | ----- | 2.00 |
| 11 | | | 14 | −23 | | ----- | 8.00 |
| 11 | Open charcoal pan | (?) | 11 | −18 | do | ----- | 3.00 |
| 11 | | | 14 | −24 | | ----- | 9.00 |
| 12 | Charcoal pan | (?) | 14 | −18 | Feb. 8 | ----- | 2.00 |
| 13 | | | 16 | −22 | | ----- | 7.00 |
| 9 | Stove with chimney | (?) | 12 | −18 | do | ----- | 3.00 |
| 12 | | | 19 | −23 | | ----- | 8.00 |

[a] Compiled by the North Manchurian Plague Prevention Service.

TABLE I.—*Temperature observations in*

| No. of observation. | Variety of dwelling. | Description of building. | Approximate size of room. | | |
|---|---|---|---|---|---|
| | | | Length. | Breadth. | Height. |
| | | | *Feet.* | *Feet.* | *Feet.* |
| 23 a<br>b | Native inn | Earth floor; kangs on both sides of room, lighted once every evening. | 19 | 19 | 9 |
| 24 a<br>b | do | Earth floor; kangs on both sides, burned once every evening; paper windows. | 16 | 19 | 9 |
| 25 a<br>b | do | Earth floor; kangs on both sides; paper window on one side. | 21 | 19 | 9 |
| 26 a<br>b | do | Earth floor; kangs on both sides | 19 | 19 | 9 |
| 27-a | Native theater | Wooden floor; all windows on top; natural vent. | 85 | 90 | 65 |
| 28 a<br>b | Native inn, room A. | Earth floor; kangs on both sides, lighted once every evening; burn straw; 2 paper windows; dome-shaped roof. | 19 | 19 | 9 |
| 29 a<br>b | Native inn, room B. | do | 19 | 19 | 9 |
| 30 a<br>b | Native inn, room A. | do | 19 | 19 | 9 |
| 31 a<br>b | Native inn, room B. | do | 19 | 19 | 9 |
| 32 | Native theater | Wooden floor; all windows on top; natural vent. | 85 | 90 | 65 |
| 33-a | Private house, room A. | Earth floor; kangs on both sides; 2 paper windows. | 9 | 14 | 7 |
| 34-a | Private house, room B. | Earth floor; kangs on both sides; 2 paper windows; dome-shaped roof. | 19 | 19 | 9 |
| 35-a | Carpenter shop | Earth floor; kangs on both sides; 1 window, papered. | 19 | 12 | |
| 36-a | Cake shop | do | 19 | 9 | 7 |
| 37-a | Private house | do | 19 | 9 | |
| 38 a<br>b | Lodging house | Earth floor; 6 paper windows | 33 | 19 | |
| 39 a<br>b | Eating house | Earth floor; 2 paper windows; kangs on either side. | 16 | 16 | 7 |
| 40 | Private house | do | 21 | 21 | 7 |
| 41 | do | do | 23 | 9 | 7 |
| 42 | do | Earth floor; kangs on both sides; 2 paper windows. | 9 | 16 | 9 |
| 43 | do | Earth floor; kangs on both sides; 1 window. | 16 | 9 | 12 |
| 44 | Small inn | Earth floor; kangs on both sides; 1 window; brick wall. | 9 | 19 | 7 |
| 45 | Private house | Plank floor; kang on one side; glass windows. | | | |

*Fuchiatien (Harbin)*—Continued.

| Occupants at the time. | How heated. | Occurrence or nonoccurrence of plague. | Temperature. | | Date. | Time of day taken. | |
|---|---|---|---|---|---|---|---|
| | | | Inside of room. | Outside of room. | | | |
| | | | °*C.* | °*C.* | 1913. | *a. m.* | *p. m.* |
| 12 | Charcoal pan; no chimney. | (?) | 4 | —14 | Feb. 9 | ------ | 1.00 |
| 11 | | | 2 | —23 | Feb. 10 | 2.00 | ----- |
| 7 | Charcoal stove with chimney. | (?) | 8 | —15 | Feb. 9 | ------ | 12.30 |
| 7 | | | 5 | —23 | Feb. 10 | 2.00 | ----- |
| 16 | Coal stove with chimney. | (?) | 11 | —15 | Feb. 9 | ------ | 3.00 |
| 18 | | | 13 | —23 | Feb. 10 | 12.30 | ----- |
| 12 | Coal stove ------------- | (?) | 8 | —15 | Feb. 9 | ----- | 3.00 |
| --------- | | | 14 | —23 | | ----- | 12.00 |
| [a]900 | 2 big brick stoves, each 6 by 3 by 3 feet. | Yes, many ------ | 11 | —22 | Feb. 9 | ------ | 11.00 |
| 15 | 1 charcoal pan without light; 1 burning. | (?) | 10 | —10 | Feb. 10 | 11.00 | ----- |
| 13 | | | 5 | —20 | Feb. 11 | 2.00 | ----- |
| 7 | Only 1 charcoal pan, unlighted. | (?) | 5 | —10 | Feb. 10 | 11.30 | ----- |
| 16 | | | 12 | —20 | Feb. 11 | 2.00 | ----- |
| 20 | No stove or pan, but kangs on both sides. | (?) | 12 | —10 | Feb. 10 | ------ | 12.30 |
| 16 | | | 11 | —20 | Feb. 11 | 1.30 | ----- |
| 10 | 1 charcoal pot, no chimney; burning. | (?) | 11 | —10 | Feb. 10 | ------ | 12.30 |
| 11 | | | 10 | —20 | Feb. 11 | 1.30 | ----- |
| 650 | 2 big brick stoves, each 6 by 3 by 3 feet. | Yes ------------ | (b) | — 6 | Feb. 16 | ------ | 2.00 |
| 8 | Charcoal pan, lighted -- | -----do ---------- | 10 | — 7 | Feb. 11 | ------ | 4.00 |
| 11 | Charcoal pan, but without fire. | -----do ---------- | 11 | — 7 | ----do ---- | ------ | 4.00 |
| 5 | No stove or charcoal pan whatever. | -----do ---------- | 0 | — 9 | ----do ---- | ------ | 5.00 |
| 2 | Kang only ------------ | -----do ---------- | 6 | — 6 | ----do ---- | ------ | 2.00 |
| 4 | -----do ---------------- | -----do ---------- | 4 | — 6 | ----do ---- | ------ | 2.30 |
| 8 | Kangs; charcoal pan with fire. | Reported to have occurred. | 10 | — 7 | Feb. 12 | ------ | 1.00 |
| 18 | | | 6 | —19 | Feb. 13 | 1.30 | ----- |
| 5 | Charcoal pan, burning - | Yes ------------ | 1 | — 7 | Feb. 12 | ------ | 2.00 |
| 5 | | | 5 | —19 | Feb. 13 | 1.30 | ----- |
| 13 | Stove with chimney; not lighted. | -----do ---------- | 12 | — 7 | Feb. 12 | ------ | 2.00 |
| 4 | Charcoal pan, burning - | -----do ---------- | 8 | — 6 | ----do ---- | ------ | 3.00 |
| 8 | -----do ---------------- | Yes; many ------ | 13 | — 7 | Feb. 13 | ------ | 4.00 |
| 2 | Stove with chimney; no fire. | -----do ---------- | 14 | — 6 | ----do ---- | ------ | 5.00 |
| 2 | Stove with chimney; burning. | Yes; over 10 persons. | 16 | — 6 | ----do ---- | ------ | 3.00 |
| 6 | Chimney stove with fire. | ---------------- | 18 | — 7 | ----do ---- | ------ | 4.00 |

[a] About.

[b] Downstairs, 9° C.; upstairs, 11° C.

TABLE II.—*Temperature obser*

| No. of observation. | Variety of dwelling. | Description of building. | Approximate size of room. | | |
|---|---|---|---|---|---|
| | | | Length. | Breadth. | Height. |
| | | | *Feet.* | *Feet.* | *Feet.* |
| 1 | Chinese hotel | Plank floor; 1 glass window at the back wall; 1 entire glass window in front wall open to the yard; a kang inside room. | 22 | 11 | 12 |
| 2 | Chinese theater | Plank floor; a U-shaped amphitheater facing the stage; glass windows on all sides, but these always closed in cold weather; about 600 seats. | 150 | 70 | 50 |
| 3[d] | Small hawker's dwelling. | Earth floor; opposite kangs; dark; 2 paper windows, each about 2 feet square. | 13 | 19 | 8 |
| 4[d] | Hawker's shop | Earth floor; 1 kang; 2 skylights; 2 paper windows; part of room used for cooking. | 19 | 13 | 6 |
| 5[d] | Coolie hut | Earth floor; one side of the room used; kaoliang (millet) stalks as a partition; 2 paper windows; 1 kang. | 19 | 13 | 8 |
| 6 | Public bath | Cement floor; glass windows in front wall; wooden benches along all sides of wall; bright but air-tight. | 34 | 20 | 11 |
| 7 | Chinese inn | Earth floor; 1 kang; wooden partition dividing room into 2 portions; 2 paper windows behind kang. | 10 | 9 | 8 |
| 8 | do | Plank floor; paper window in front; 2 kangs opposite each other, 1 was heated. | 23 | 12 | 10 |
| 9 | Chinese low-class inn. | Earth floor; damp; dark; 3 kangs; paper window; dome-shaped roof. | 18 | 22 | 10 |
| 10 | Private residence. | Earth floor; dark and damp; 2 kangs; millet-stalk roof, conical shape; paper window; half room used as kitchen. | 17 | 21 | 9 |
| 11 | Chinese inn | Plank floor; paper window; walls lined with paper; have not been torn down since the epidemic, but have had a little alteration since then. | 20 | 13[c] | 9 |
| 12 | Police station | Earth floor; opposite kangs; paper windows; damp and dark; mud wall; millet-stalk roof. | 37 | 18 | 10 |
| 13 | Chinese low-class inn. | Earth floor; opposite kangs; street wall has 2 paper windows about 3 feet square; 1 window at back wall; paper ceiling; mud wall. | 16 | 16 | 9 |

[a] Compiled by the North Manchurian Plague Prevention Service.
[b] Medical officer himself.
[c] About.
[d] The houses of observations 3, 4, and 5 are situated 2 miles from Changchun, so a second observation was not made.

*vations in Changchun.*[a]

| Occupants at the time. | How heated. | Occurrence or nonoccurrence of plague. | Temperature. |  | Date. | Time of day taken. |  |
|---|---|---|---|---|---|---|---|
|  |  |  | Inside of room. | Outside of room. |  |  |  |
|  |  |  | °C. | °C. | 1913. | a. m. | p. m. |
| [b]1 | 1 kang; charcoal pan burning; a stove with chimney extending from next room. | No; built after the epidemic. | 7 | −3 | Feb. 21 | 1.00 | ----- |
| 1 |  |  | 5 | −3 |  | 6.00 | ---- |
| [c]370 | 3 stoves with chimney at corners of ground floor. | do | 5 | −7 | do | ----- | 9.00 |
| 7 | 1 small charcoal pan kept burning. | Yes | −3 | −7 | Feb. 22 | ----- | 3.00 |
| 7 | Earthen pot burning charcoal. | do | 1 | −7 | do | ----- | 3.00 |
| [e]13 | No stoves; kang lighted once or twice a day for cooking. | do | 1 | −8 | do | ----- | 3.00 |
| 12 | Chimney stove burning coal. | Yes; over 30 | [f]23 | −8 | do | ----- | 4.00 |
| 10 | Stove burning coal in one of the rooms. | Yes, but not this room; the infected room is now locked up. | 13 | −8 | do | ----- | 4.30 |
| 14 |  |  | 14 | −15 |  | ----- | 11.00 |
| 10 | Stove with chimney; burning coal. | No | 21 | −8 | do | ----- | 5.15 |
| 7 |  |  | 17 | −15 | Feb. 23 | ----- | 12.30 |
| 11 | Stove with chimney; burning. | Yes | 16 | −8 | do | ----- | 12.30 |
| 20 |  |  | 16 | −19 | Feb. 24 | 2.00 | ----- |
| 5 | No stove; but a square brick stove for cooking. | Yes; all in house were carried off by plague. | 0 | −8 | Feb. 23 | ----- | 1.00 |
| 6 |  |  | 1 | −19 | Feb. 24 | 2.00 | ----- |
| 6 | Stove with chimney extending into it from next room. | Yes | 9 | −8 | Feb. 23 | ----- | 1.00 |
| 10 |  |  | 9 | −20 | Feb. 24 | 2.00 | ----- |
| 18 | No stove whatever; kangs burned twice a day. | Yes; 24 out of 25 persons. | 11 | −5 | do | ----- | 2.00 |
| 29 |  |  | 3 | −14 | Feb. 25 | 6.00 | ----- |
| 11 | Only 2 kangs that receive warmth from 2 cooking stoves outside. | Yes | 5 | −5 | Feb. 24 | ----- | 2.30 |
| 16 |  |  | 4 | −14 | Feb. 25 | 5.50 | ----- |

[e] All on the kang for warmth.
[f] Taken at corner of room.

TABLE II.—*Temperature observations*

| No. of observation. | Variety of dwelling. | Description of building. | Approximate size of room. | | |
|---|---|---|---|---|---|
| | | | Length. | Breadth. | Height. |
| | | | *Feet.* | *Feet.* | *Feet.* |
| 14 | Chinese low-class inn. | Earth floor; 3 kangs like a ∩ in shape; opposite walls both were paper-lined; 3 square brick stoves attached to each of the kangs; old and damp. | 32 | 33 | 11 |
| 15 | do | Earth floor; opposite kangs; paper window on street side; old and wet. | 20 | 17 | 9 |
| 16 | Chinese inn | Earth floor; ∩-shaped kangs; 1 paper window; 1 brick stove for cooking; kaoliang-stalk roof. | 34 | 20 | 10 |
| 17 | Wagonmaker | Earth floor; 3 paper windows on one side; damp; 3 flat cooking stoves; mud wall. | 24 | 22 | 10 |

*in Changchun*—Continued.

| Occupants at the time. | How heated. | Occurrence or nonoccurrence of plague. | Temperature. | | Date. | Time of day taken. | |
|---|---|---|---|---|---|---|---|
| | | | Inside of room. | Outside of room. | | | |
| | | | °*C.* | °*C.* | 1913. | *a. m.* | *p. m.* |
| 13 | Only the 3 brick stoves for cooking. | Yes; about 3 or 4 persons lost lives here. | 5 | — 9 | Feb. 24 | ----- | 3.00 |
| 24 | | | 3 | —14 | Feb. 25 | 6.00 | ----- |
| 5 | An open charcoal pan | Yes | 5 | — 5 | Feb. 24 | ----- | 3.30 |
| 20 | 1 charcoal pan burning | do | 8 | — 6 | Feb. 25 | ----- | 5.00 |
| 20 | | | 6 | —18 | Feb. 26 | 2.00 | ----- |
| 25 | Only the 3 stoves attached to the kangs burned when cooking. | do | 9 | — 6 | Feb. 25 | ----- | 5.00 |
| 23 | | | 9 | —18 | Feb. 26 | 3.00 | ----- |

It is seen from Table II that plague cases were known to have occurred in all but 3 of the houses investigated in Changchun and that temperatures of 6° C. or less were recorded in 11 of the 17 houses investigated.

Consequently, I am still of the opinion that atmospheric temperature is an important factor in determining the spread or failure to spread of pneumonic plague.

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